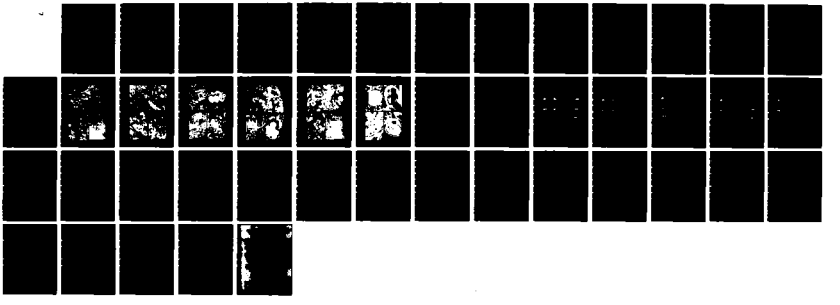
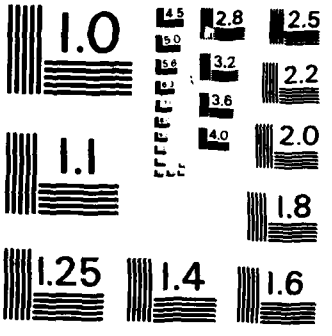
AD-A142 398 RESPONSES OF CHOLINERGIC AND NONCHOLINERGIC RENSHAW CELL RECEPTORS AFTER..(U) IOWA STATE UNIV AMES W G VAN METER ET AL. JUL 83 DAMD17-80-C-0106 1/1

UNCLASSIFIED F/G 6/16 NL





MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS - 1963 - A

(12)

AD_____

RESPONSES OF CHOLINERGIC AND NONCHOLINERGIC RENSHAW CELL RECEPTORS AFTER ACUTE AND CHRONIC EXPOSURE TO ANTICHOLINESTERASES (U)

ANNUAL SUMMARY REPORT

WILLIAM G. VAN METER, Ph. D.
KARIN C. SIKORA-VAN METER, Ph. D.

August 1983

Supported by
U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701

Contract No. DAMD17-80-C-0106
Iowa State University
Ames, Iowa 50011

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER	2. GOVT ACCESSION NO. AD-A142	3. RECIPIENT'S CATALOG NUMBER 398
4. TITLE (and Subtitle) RESPONSES OF CHOLINERGIC AND NONCHOLINERGIC RENSHAW CELL RECEPTORS AFTER ACUTE AND CHRONIC EXPOSURE TO ANTICHOLINESTERASES		5. TYPE OF REPORT & PERIOD COVERED Annual 15 May 1981 - 14 May 1982
		6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) William G. Van Meter Karin C. Sikora-Van Meter		8. CONTRACT OR GRANT NUMBER(s) DAMD17-80-C-0106
9. PERFORMING ORGANIZATION NAME AND ADDRESS Iowa State University Ames, Iowa 50010		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS 61102A.3M161102BS10.EA.085
11. CONTROLLING OFFICE NAME AND ADDRESS US Army Medical Research and Development Command Fort Detrick, Frederick, Maryland 21701		12. REPORT DATE July 1983
		13. NUMBER OF PAGES 42
14. MONITORING AGENCY NAME & ADDRESS(if different from Controlling Office)		15. SECURITY CLASS. (of this report) Unclassified
		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

APPROVED FOR PUBLIC RELEASE, DISTRIBUTION UNLIMITED

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Renshaw Cell, Renshaw field potentials, Renshaw unit potentials, DFP, Cat, Ventral horn, Spinal cord, Chronic DFP, Ultrastructure

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

The fine structural changes in the most ventral regions of the ventral horn of spinal segment L7 have been studied in cats after single and chronic low dose exposure to diisopropylfluorophosphate (DFP). Motoneurones of the chronically treated animals show an increase in the number of lysosomes, neurofilaments, and vesicle-like structures. A large number of coated vesicles is observed within axons and axon terminals of both acute and chronically treated animals. Mohrphological evidence of axon and terminal degeneration is seen only in chronically treated animals. Degeneration is

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

20. Abstract

revealed by the presence of membrane-bound structures, glycogen granules, lamellar and dense lamellar bodies, increased amounts of smooth axonal endoplasmic reticulum (sAER), neurofilaments and accumulation of microtubules. Axonal degeneration in the ventral horn of L7 after chronic low dose exposure to DFP is suggested to be a retrograde process starting at the presynaptic terminals.

Effects following intravenous administration of anticholinesterase and anticholinergic drugs on Renshaw cell unit potentials (RUP) have been studied in the spinal cord of cats anaesthesized with DIAL anaesthesia. The RUP are evoked by antidromic stimulation (1-2 Hz @ 2x threshold) of L7 ventral roots and recorded by conventional means with single barrel glass micropipettes filled with 2.7 M NaCl (tip diameter 1.0-1.6 microns, resistance of 2.0-2.4 megohms). Neither acute nor chronic administration of DFP (total doses of 2.0 mg/Kg i.v. and 2.8 mg/Kg s.c. respectively) markedly affects the response to 1 or 2 Hz stimulation. However, the onset of post tetanic depression (PTD) is consistently more rapid than in the untreated cats. Mecamylamine (1.0 mg/Kg i.v.) markedly antagonises the response to 1-2 Hz stimulation and only the first 2 spikes remain. This effect can not be reversed by DFP (2.5 mg/Kg i.v.) nor by excess ACh via 20 Hz stimulation. Atropine sulfate (1.0 mg/Kg i.v.) fails to antagonise the depressant effect of Eserine (500 mcgm i.v.) on RUP.

Renshaw cell field potentials (RFP) are inhibited during the first 30-60 seconds of 20 Hz stimulation and the frequency and number of RFP are reduced during the first 1-2 minutes of recovery with recovery occuring within 3-5 minutes after 20 Hz stimulation. Eserine (50 and 100 mcgm/Kg i.v.) enhances responses to 20 Hz stimulation. Recovery is not observed until 3 minutes after cessation of 20 Hz stimulation. Mecamylamine (1.0 mg/Kg i.v.) significantly reduces the RFP similar to that seen with RUP with only the first two potentials remaining even in the presence of atropine (1.0 mg/Kg i.v.). Amphetamine (0.5 and 1.0 mg/Kg i.v.) was given to assess possible interaction between cholinergic and adrenergic mechanisms. No effect is seen and amphetamine fails to potentiate subeffective doses of Eserine (20 mcgm/Kg i.v.). The data are interpreted to indicate a muscarinic and nicotinic component to the RFP and a possible third component as revealed by the persistence of the first two potentials in the presence of atropine and of mecamylamine given together.

A-1

AD_____

RESPONSES OF CHOLINERGIC AND NONCHOLINERGIC RENSHAW CELL RECEPTORS AFTER ACUTE AND CHRONIC EXPOSURE TO ANTICHOLINESTERASES (U)

ANNUAL SUMMARY REPORT

WILLIAM G. VAN METER, Ph. D.
KARIN C. SIKORA-VAN METER, Ph. D.

August 1983

Supported by
U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701

Contract No. DAMD17-80-C-0106
Iowa State University
Ames, Iowa 50011

UNCLASSIFIED

ABSTRACT:

The fine structural changes in the most ventral regions of the ventral horn of spinal segment L7 have been studied in cats after single and chronic low dose exposure to diisopropylfluorophosphate (DFP). Motoneurones of the chronically treated animals show an increase in the number of lysosomes, neurofilaments and vesicle-like structures. A large number of coated vesicles is observed within axons and axon terminals of both acute and chronically treated animals. Morphological evidence of axon and terminal degeneration is seen only in chronically treated animals. Degeneration is revealed by the presence of membrane-bound structures, glycogen granules, lamellar and dense lamellar bodies, increased amounts of smooth axonal endoplasmic reticulum (sAER), neurofilaments and accumulation of microtubules. Axonal degeneration in the ventral horn of L7 after chronic low dose exposure to DFP is suggested to be a retrograde process starting at the presynaptic terminals.

Effects following intravenous administration of anticholinesterase and anticholinergic drugs on Renshaw cell unit potentials (RUP) have been studied in the spinal cord of cats anaesthesized with DIAL anaesthesia. The RUP are evoked by antidromic stimulation (1-2 Hz @ 2x threshold) of L7 ventral roots and recorded by conventional means with single barrel glass micropipettes filled with 2.7 M NaCL (tip diameter 1.0-1.6 microns, resistances of 2.0-4.0 megohms). Neither acute nor chronic administration of DFP (total doses of 2.0 mg/Kg i.v. and 2.8 mg/Kg s.c. respectively) markedly affects the response to 1 or 2 Hz stimulation. However, the onset of post tetanic depression (PTD) is consistently more rapid than in the untreated cats. Mecamylamine (1.0 mg/Kg i.v.) markedly antagonises the response to 1-2 Hz stimulation and only the first 2 spikes remain. This effect can not be reversed by DFP (2.5 mg/Kg i.v.) nor by excess ACh via 20 Hz stimulation. Atropine sulfate (1.0 mg/Kg i.v.) fails to antagonise the depressant effect of Eserine (500 mcgm i.v.) on RUP.

Renshaw cell field potentials (RFP) are inhibited during the first 30-60 seconds of 20 Hz stimulation and the frequency and number of RFP are reduced during the first 1-2 minutes of recovery with recovery ocuring within 3-5 minutes after 20 Hz stimulation. Eserine (50 and 100 mcgm/Kg i.v.) enchances responses to 20 Hz stimulation. Recovery is not observed until 3 minutes after cessation of 20 Hz stimulation. Mecamylamine (1.0 mg/Kg i.v.) significantly reduces the RFP similar to that seen with RUP with only the first two potentials remaining even in presence of atropine (1.0 mg/Kg i.v.). Amphetamine (0.5 and 1.0 mg/Kg i.v.) was given to assess possible interaction between cholinergic and adrenergic mechanisms. No effect is seen and amphetamine fails to potentiate subeffective doses of Eserine (20 mcgm/Kg i.v.). The data are interpreted to indicate a muscarinic and nicotinic component to the RFP and a possible third component as revealed by the persistence of the first two potentials in the presence of atropine and of mecamylamine given together.

FOREWARD

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

TABLE OF CONTENTS

INTRODUCTION:

Numerous light (Abrahams et al. (1957), Burt and Silver (1973), Butcher and Bilezikjian (1975), Butcher et al. (1975), Koelle (1954), Parent et al. (1977), and VanMeter and Karczmar (1978)) and electron microscopic studies (Burt (1971), Kaiya et al. (1980), Shimizu and Ishii (1966), and Somogyi and Chubb (1976)) have used acute exposure to organophosphate cholinesterase inhibitors (ChEI's), in particular DFP (Koelle and Gilman (1949), Poirier et al. (1977), Toth et al. (1980)), to study the organization of cholinergic systems in the brain and spinal cord and to demonstrate acetyl- and butryl- cholinesterase inhibition (AChEI and BuChEI, respectively) in these systems. In addition, morphological evidence of myopathy in skelatal muscle has been seen after exposure to DFP, soman, paraoxon, tabun, TCP, or TCOP (Ariens et al. (1969), Engel et al. (1974), Fischer (1970), Glazer et al. (1978), Laskowski et al. (1975), Laskowski et al. (1977), Preusser (1967), and Toth et al. (1980)). Delayed neurotoxicity has been demonstrated after acute exposure to TCP and TCOP in brain and spinal cord (Ahmed and Gless (1968), Ahmed (1973), Bischoff (1970), Cavanagh (1954), Gless and Janzik (1965) and Prineas (1969)). Furthermore, acute TCP intoxication has been shown to alter mitochondria in spinal ganglia (Ahmed and Gless (1977)) and to show degenerative changes in spinal cord synapses (Ahmed (1971), Bischoff (1970), Gless and Janzik (1965), and Prineas (1969)). On the other hand, tolerance to behavioral effects of DFP and soman is seen after chronic administration (Brodeur and Dubois (1964), McPhillips (1969), Sterri et al. (1980), for review see Russell et al. (1975)). Also chronic exposure to paraoxon shows a progressive myopathy in skeletal muscle (Fenichel et al. (1972)). Since cholinergic transmission in the CNS has been demonstrated to exist between the motoneurones and Renshaw cells located in the ventral portion of lumbar spinal segment 7 (L7) of Rexed's Lamina VII (Eccles et al. (1954)) this area of L7 has been chosen to study changes in the fine structure of cats acutely and chronically treated with DFP. This preliminary report presents data on fine structural changes in the cat L7 (VII) with emphasis on those effects due to chronic cholinesterase inhibition from daily administration of DFP.

Repetitive (20Hz) antidromic stimulation of L7 ventral spinal roots presumably models an increase in ACh at the motoneurone axon collateral - Renshaw cell synapse. Annual Report dated July 1983 describes the characteristic responses of Renshaw cell unit (RUP) and field (RFP) potentials to such stimulation. The present report is a description of the effects of acute and chronic exposure to DFP, of acute exposure to Eserine and to amphetamine as well as studies on antagonism with mecamylamine and atropine sulfate on the RUP and RFP.

MATERIAL AND METHODS

A. MORPHOLOGY

After conditioning for 3 weeks, male or spayed female cats weighing 2.0 to 3.5kg were housed separately and allowed food and water ad libitum. Chronic administration of DFP (0.1 to 0.75mg/kg) or its vehicle polyethylene glycol (PEG, 0.1ml/kg) was given subcutaneously every 24h for a period of 3 to 21 days (Table 1). Cats were weighed daily and observed for symptoms of toxicity.

Prior to removal of tissue samples, cats were anesthetized with DIAL (80mg/kg, i.p.) supplemented with sodium pentobarbital as required and blood pressure was supported by i.v. infusion of 5% glucose in 50% lactated Ringer solution. Atropine methyl nitrate (0.3mg/kg, i.m.) and penicillin G (120,000 units i.m.) were routinely administered to all animals. A laminectomy was performed from sacral segment 1 (S1) to lumbar segment 1 (L1), the dura opened and ipsilateral dorsal and ventral roots of S1, L7 and L6 ligated and transected. The cord was transected at L1 following administration of 2% lidocaine.

Tissue samples (dorsal and ventral spinal roots as well as 2-3mm sections of the rostral portions of spinal segments S1, L7 and L6) were obtained from cats either without additional experimentation or after electrophysiological investigation of ventral horn interneurons. The tissue samples were fixed by immersion in 5% phosphate buffered (0.1M, pH 7.4) glutaraldehyde.

After 24 hours of immersion fixation, the tissue was trimmed into 0.5 to 1mm thick sections, followed by postfixation in 1% OsO_4 and en block staining with 2% uranyl acetate. The tissue was dehydrated and embedded in either Spurr or a Poly-Bed812/Araldite mixture. Ultrathin serial sections were taken from the most ventral 1.5mm of the ventral horn of L7, stained with uranyl acetate and lead citrate with an LKB Ultrastainer prior to examination in a Hitachi 12A electron microscope.

B. ELECTROPHYSIOLOGICAL/NEUROPHYSIOLOGICAL:

Data were obtained from adult (2.0-3.0 Kg) male or spayed female mongrel cats anaesthetised with DIAL compound (diallyl barbituric acid and urethane) in doses of 80.0 mg/Kg i.p. supplanted with minimal amount of pentobarbital as needed. Animals were allowed to breathe spontaneously with arterial blood pressure monitored and kept above 60 torr by i.v. infusion of 5% dextrose in saline as required. Expired carbon dioxide was monitored throughout by a Beckman LB2 carbon dioxide monitor. Penicillin G (3000 units i.m.) was administered since experimental procedures routinely exceeded 12 hours. A laminectomy of the lumbo-sacral spinal cord was performed from sacral segment 1 (S-1) to lumbar segment 1 (L-1) or thoracic segment 13 (T-13). THe preparation was spinalised at T-13/L-1 after local injection of 2% procaine. Ipsilateral dorsal and ventral spinal roots (S-1, L-7, L-6) were separated and transected prior to being placed on bipolar platinum stimulating electrodes for ortho and antidromic stimulation. After surgical preparation and recovery (1-2 hrs.) the cats were placed in a Kopf spinal investigation unit and the cord covered with a deep layer of medicinal paraffin held over the back by retaining sutures in the skin flaps. Body and paraffin temperature were maintained above 35 degrees celsius by heating pads and infrared heating lamps.

Selection of the dorsal and ventral spinal roots for stimulation was facilitated by means of a rotary switching device. Extracellular Renshaw cell unit and field potentials were recorded by conventional means from 2.7M NaCl filled glass micropipettes with tip diameters of 1.0-1.5 micron and resistances, after beveling, of 2.0-4.0 megohms. Data was obtained on film or was recorded on FM tape for subsequent analysis. Renshaw cell unit potentials (RUP) were identified by their characteristic discharge to supramaximal antidromic ventral root activation and could be

recorded in an extremely limited area, usually the ventral most portion of L-7 as identified histologically after experiments (cf Eccles et al 1954, and Willis et al 1969). Renshaw cell field potentials (RFP) were identified similarly by their characteristic discharge to supramaximal antidromic L-7 ventral root stimulation but were recorded over a much larger area of the spinal segment and showed a more oscillatory pattern than the true spike pattern as seen with the RUP. Antidromic stimulation to evoke RUP or RFP was delivered at 1 or 2 Hz during control periods or after the administration of drugs. Responses of RUP and RFP in the presence of endogenous excess acetylcholine (ACh) were obtained by repetitive stimulation of the ventral spinal roots at 20 Hz, a frequency at which motoneurone and Renshaw cell discharges are linear (Ross et al 1976). Responses (RUP and RFP) during repetitive stimulation (20 Hz) and afterwards (1 or 2 Hz) were evaluated for either acute or chronic drug effects.

Diisopropylfluorophosphate (DFP) was administered either sub cutaneously in chronically treated animals or intravenously in acute experiments in sub LD50 doses (LD50=3.0 mg/Kg) whether given singly or the accumulated amount. Eserine was selected as a centrally acting reversible cholinesterase inhibitor (CHEI) and was given acutely in amounts ranging from 20 mcgm/Kg i.v. to 500 mcgm/Kg i.v.. Antimuscarinic drug action in the Central Nervous System was obtained by atropine sulfate in doses of 0.5 and 1.0 mg/Kg. i.v. while atropine methyl nitrate in doses of 0.3 mg/Kg i.v. was used to protect the periphery of the cats from untoward responses to anticholinesterses (antiCHE). Mecamylamine (1.0 mg/Kg i.v.) was used for central antinicotinic effects. Amphetamine (0.5-1.0 mg/kg) was used not only for its action on RFP but also as a possible potentiator of the effects of Eserine on RFP (cf. VanMeter 1977).

RESULTS:

A. MORPHOLOGY

i. MOTONEURONES--The motoneurone cytoplasm contains rough endoplasmic reticulum (rER), Golgi apparatus, lysosomes, mitochondria, neurofilaments, and a round centrally located nucleus with a prominent nucleolus (Fig. 1 for detailed description see Conradi (1969 and 1976). The cisternae of rER are slightly dilated but all cell organelles have a normal appearance (Fig. 2).

Chronic but not acute administration of DFP results in an increase in lysosomal elements. Furthermore, the rER often forms clusters located towards the center of the perikaryon (Fig 3). In addition, chronic administration of 0.3 to 0.75 mg/kg DFP for 5 to 21 days often results in accumulation of membrane-bound vesicle-like structures located towards the cell periphery (Fig. 4).

An increased amount of neurofilaments throughout the cytoplasm of the neurones is observed after acute or chronic DFP administration. While glycogen particles seldom occur in motoneurones of control animals and are often located within large dendritic trunks (Fig. 5) animals treated chronically up to 19 days show an increase in glycogen particles and they accumulate in groups throughout the cytoplasm (Fig. 6) as well as dendritic trunks. In contrast, animals treated with 0.3 mg/kg DFP over 21 days do not show glycogen granules, either in the soma or in dendrites.

ii. AXONS, DENDRITES AND PRESYNAPTIC TERMINALS--Acute and chronic DFP

treatment increases the number of coated vesicles in axons (Fig. 7) as well as in axon terminals (Fig. 8). After acute exposure there are more coated vesicles in non-myelinated axons and their preterminal portions than in the presynaptic terminals. After chronic DFP administration increases in coated vesicles are seen in both axons and axon terminals.

Furthermore, chronic DFP administration often results in pronounced postsynaptic densities, occasionally seen in connection with vesicles and smooth cisternae (Fig. 9). Animals treated with 0.1 mg/kg DFP for 14 days show initial signs of degeneration in presynaptic terminals. Degeneration is represented by membrane-bound structures frequently w[illegible]h glycogen particles in apposition to them (Figs. 9 and 10). Whi[illegible]milar responses occur after chronic administration of 0.2 mg/kg DFP fo[illegible] days, more severe degeneration is observed after 0.2 mg/kg (or greater) [illegible]14 days. The presynaptic terminals show accumulation of microtubule[illegible]nd neurofilaments (Fig. 11) and others often contain many mitochondria a[illegible]ıse lamellar bodies (Fig. 12). Complete lamellation of presynaptic t[illegible]nals is present as well (Fig. 13). Treatment with 0.3 mg/kg DFP for 21 days or 0.5 mg/kg for 7 days results in an increased amount of degenerating presynaptic terminals and depletion of synaptic vesicles than observed in animals treated with lower doses of DFP (Fig. 14).

All chronically treated animals show increased evidence of various degenerative stages in non-myelinated nerve fibres. In most cases, degenerating profiles are recognized by the presence of lamellar and dense lamellar bodies (Figs. 15 and 16). Other fibres contain large amounts of filamentous material or accummulation of smooth axonal endoplasmic reticulum (Fig. 17). Occasionally profiles are observed which contain a cluster of neurofilaments with glycogen partilces in close apposition to them (Fig. 18).

Only chronic DFP administration of 0.3 mg/kg and greater causes major signs of degeneration in myelinated axons as seen by the presence of dense lamellar bodies (Fig. 19). Axons further advanced in the degenerative process contain, in addition, accumulation of mitochondria and/or filamentous material (Fig. 20). Demyelination of severely damaged axons occurs in these animals as well (Fig. 21).

FIGURE LEGENDS:

Fig. 1: Motoneurone of a control cat. N (nucleus), rER (rough endoplasmic reticulum), G (Golgi complex). Bar: 1 um.

Fig. 2: Motoneurone of a control cat with dilated cisternae of rER (arrows). Bar: 0.5 um.

Fig. 3: Motoneurone of a cat treated with 0.1mg/kg DFP for 14 days. Note the increased amount of lysosomal elements (arrows). Bar: 2um

Fig. 4: Cell periphery of a motoneurone after administration of 0.75 mg/kg DFP for 5 days. Note the large amount of membrane bound structures (arrows). Bar: 0.5um.

Fig. 5: Dendrite of a control cat with glycogen granules (arrows). Bar: 0.12 um.

Fig. 6: Motoneurone soma after treatment with 0.5mg/kg DFP over 19 days. Note the glycogen granules (arrows). Bar: 0.25 um.

Fig. 7: Axon after administration of 0.1 mg/kg DFP for 14 days. Note the coated vesicles (arrows). Bar: 0.25um.

Fig. 8: Axo-dendritic synapse in an animal treated with 0.1mg/kg DFP for 13 days. Note the coated vesicles (arrows) within the presynaptic terminal. Bar: 0.25um.

Fig. 9: Axo-somatic synapse after 0.1mg/kg DFP for 13 days. Note the pronounced postsynaptic density (pd), membrane-bound structures (mbs) in the presynaptic terminal and glycogen particles (arrows). Bar: 0.25um.

Fig. 10: C-type bouton of an animal treated with 0.1mg/kg DFP for 13 days. Note the membrane-bound structures (arrow) with glycogen particles in apposition to them. Bar: 0.25um.

Fig. 11: Presynaptic terminal in an animal treated with 0.5mg/kg DFP for 21 days with accumulation of filamentous material (arrow). Bar: 0.5um.

Fig. 12: Axo-somatic synapse after treatment with 0.2mg/kg DFP for 14 days. The degenerating presynaptic terminal containes dense lamellar bodies (arrows) and a large number of mitochondria (m). Bar: 0.25um.

Fig. 13: Presynaptic terminal in an animal after treatment with 0.3mg/kg DFP for 21 days with lamellation (arrow) and accumulation of microtubules (mt) in the center of the terminal. Bar: 0.5um.

Fig. 14: Axo-dendritic synapse of an animal treated with 0.3mg/kg DFP for 21 days. Note the small number of synaptic vesicles. Bar: 0.14um.

Fig. 15: Degenerating nerve fiber after treatment with 0.3mg/kg DFP for 21 days. m(mitochondria), lb(lamellar bodies). Bar: 0.5um.

Fig. 16: Degenerating non-myelinated fiber after treatment with 0.1mg/kg DFP for 13 days with dense lamellar bodies (arrows). Bar: 0.5um.

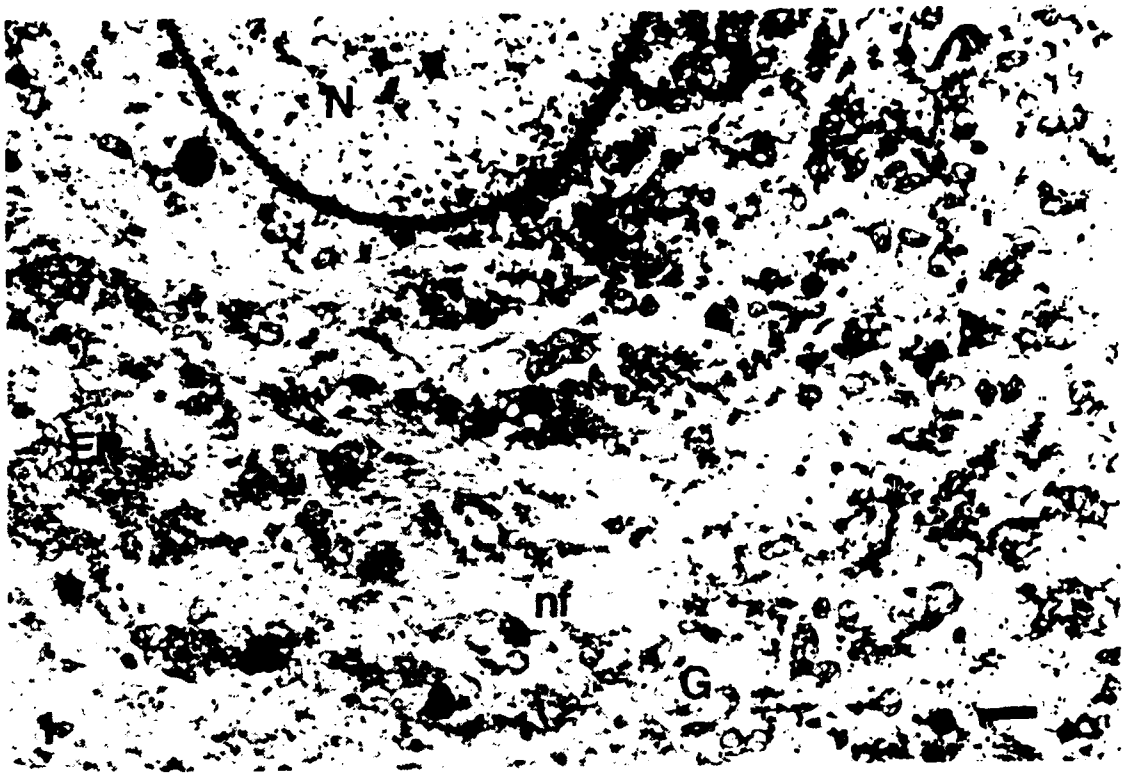
Fig. 17: Axon of an animal treated with 0.5mg/kg DFP for 7 days. Note the accumulation of smooth axonal endoplasmic reticulum (sAER). Bar: 0.25um.

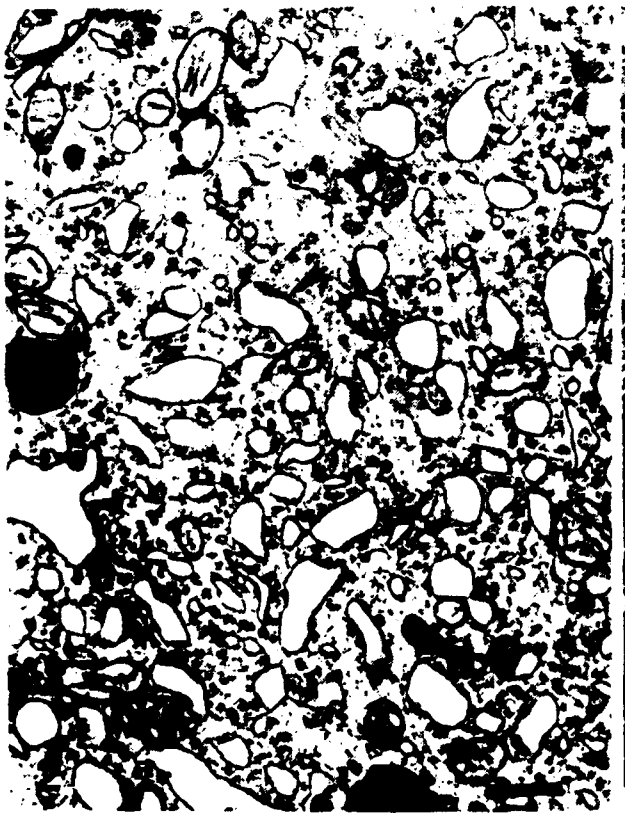
Fig. 18: Large profile of an animal treated with 0.3mg/kg DFP for 14 days. Note the accumulation of neurofilaments with glycogen particles in apposition with them. Bar: 0.5um.

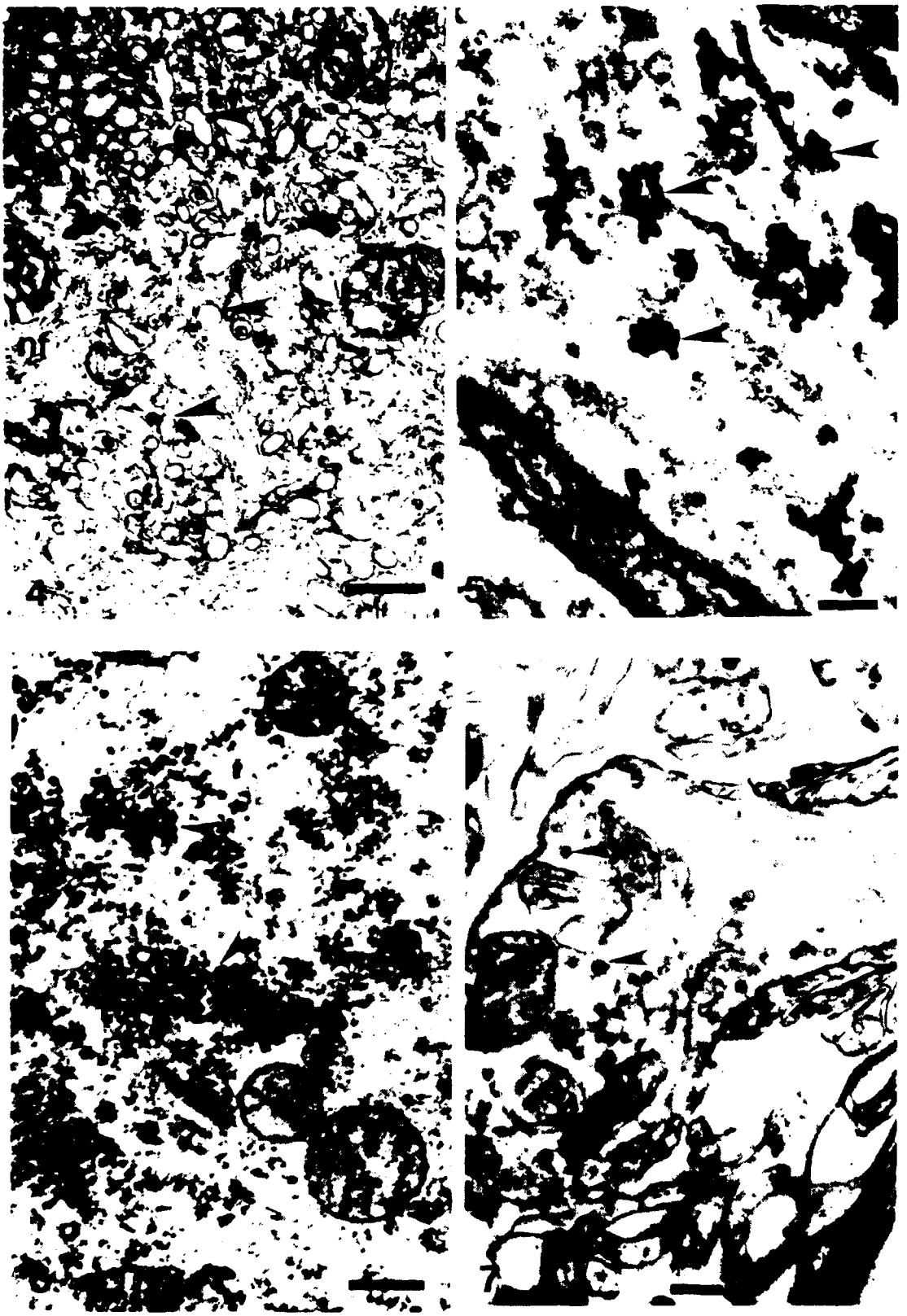
Fig. 19: Degeneration of a myelinated axon in an animal treated with 0.5mg/kg DFP for 16 days with dense lamellar bodies (arrows) as morphological indicators of degeneration. Bar: 0.25um.

Fig. 20: Myelinated axon after treatment with 0.3mg/kg DFP for 21 days. Note membrane bound structures (mbs) and dense lamellar bodies (arrows). Bar: 0.5um.

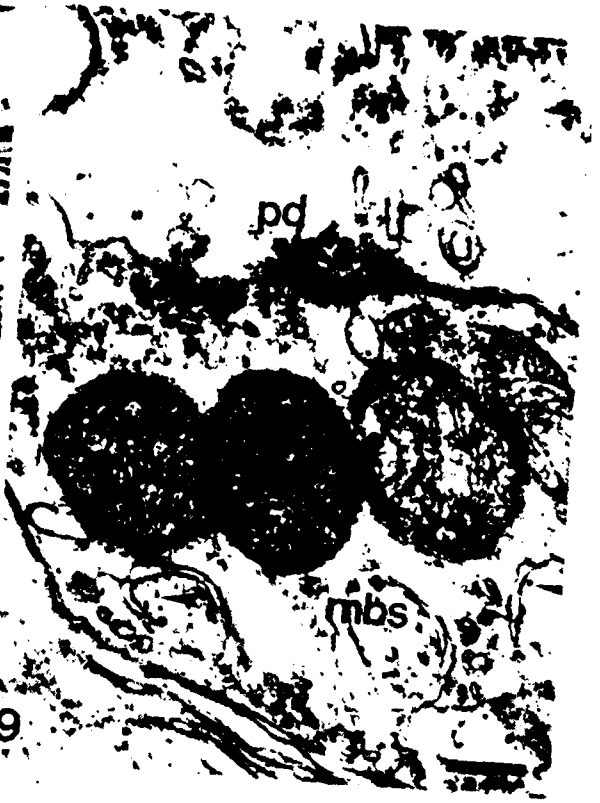
Fig. 21: Demyelination (arrow) of an axon in an animal treated with 0.3mg/kg DFP for 21 days. Note accumulation of mitochondria (m) and dense lamellar bodies as the morphological signs of degeneration in the axoplasm. Bar: 0.5um.


N
nf
G

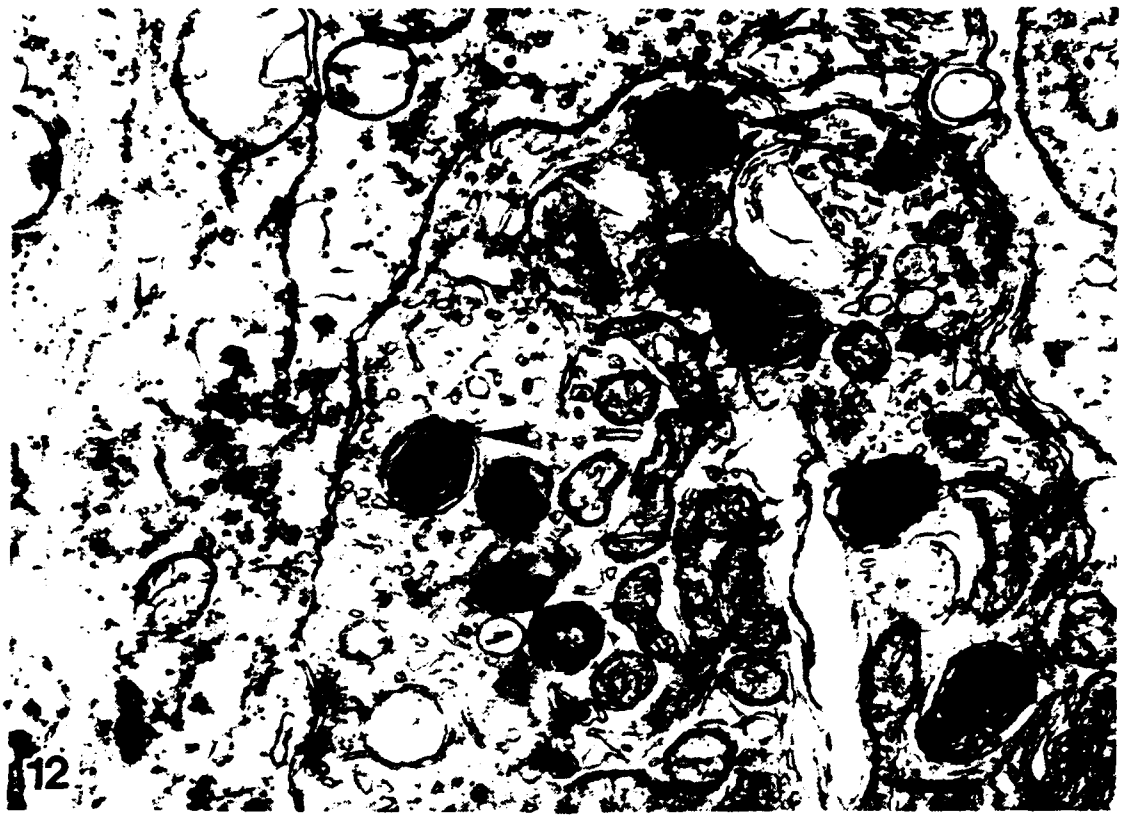


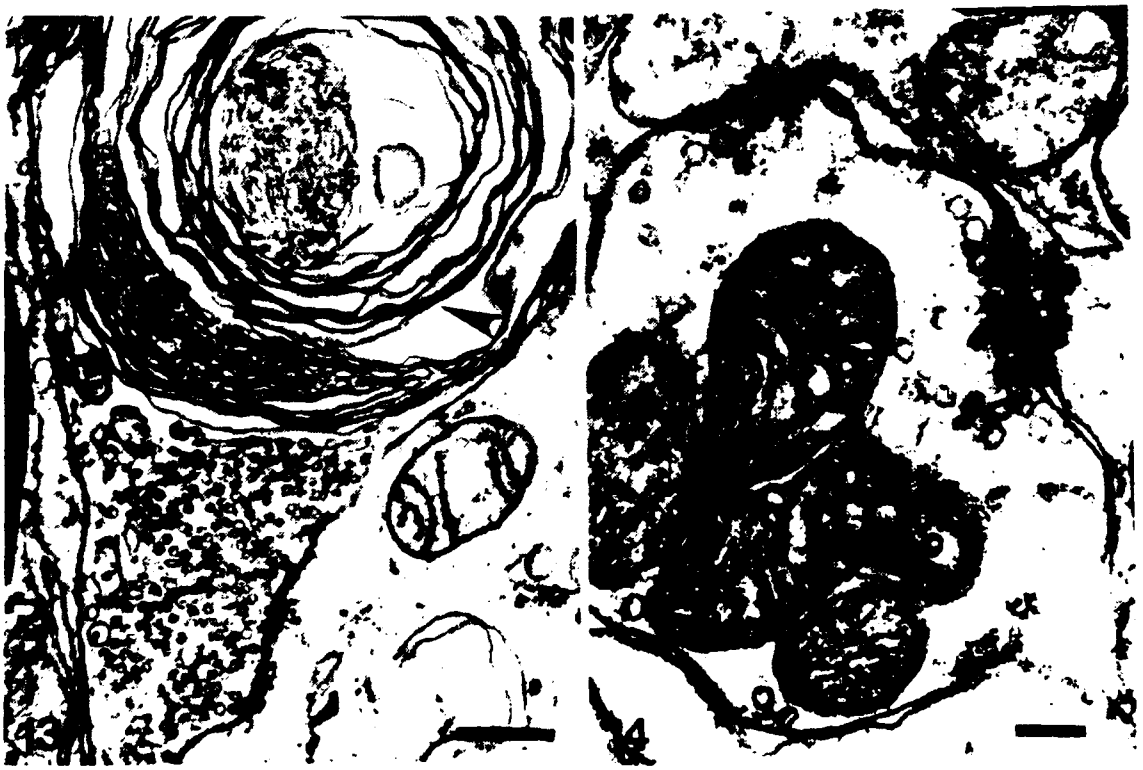


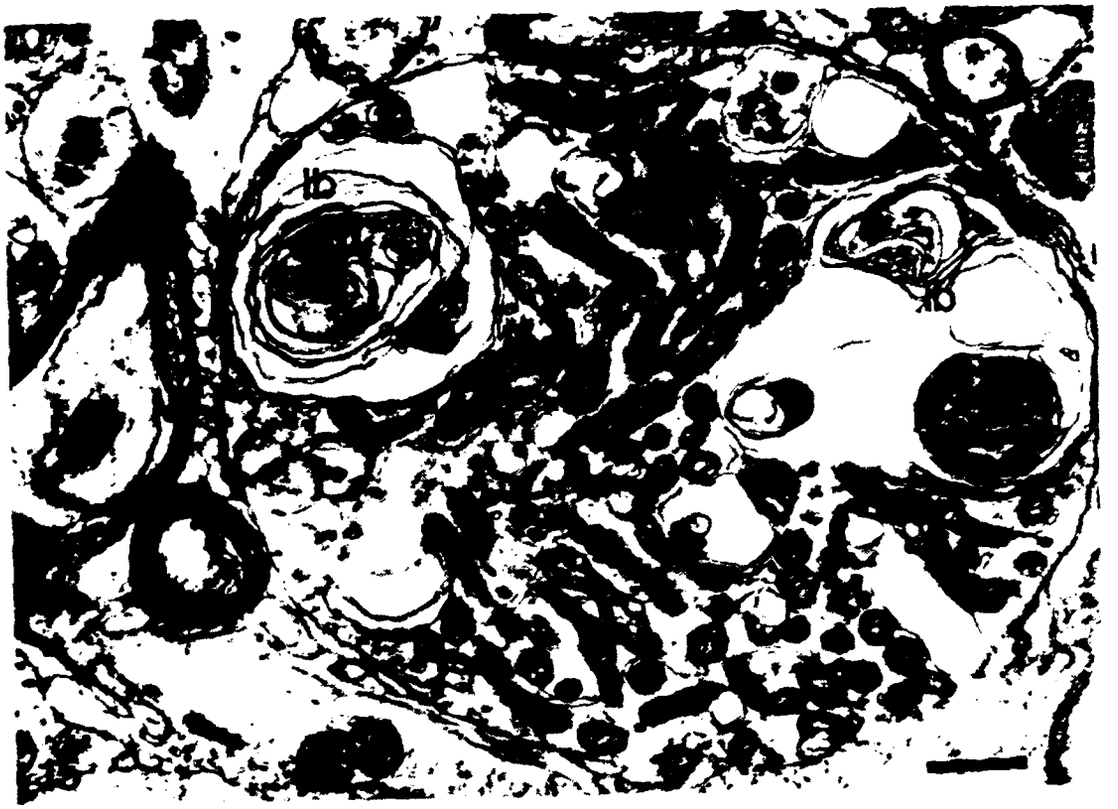
8

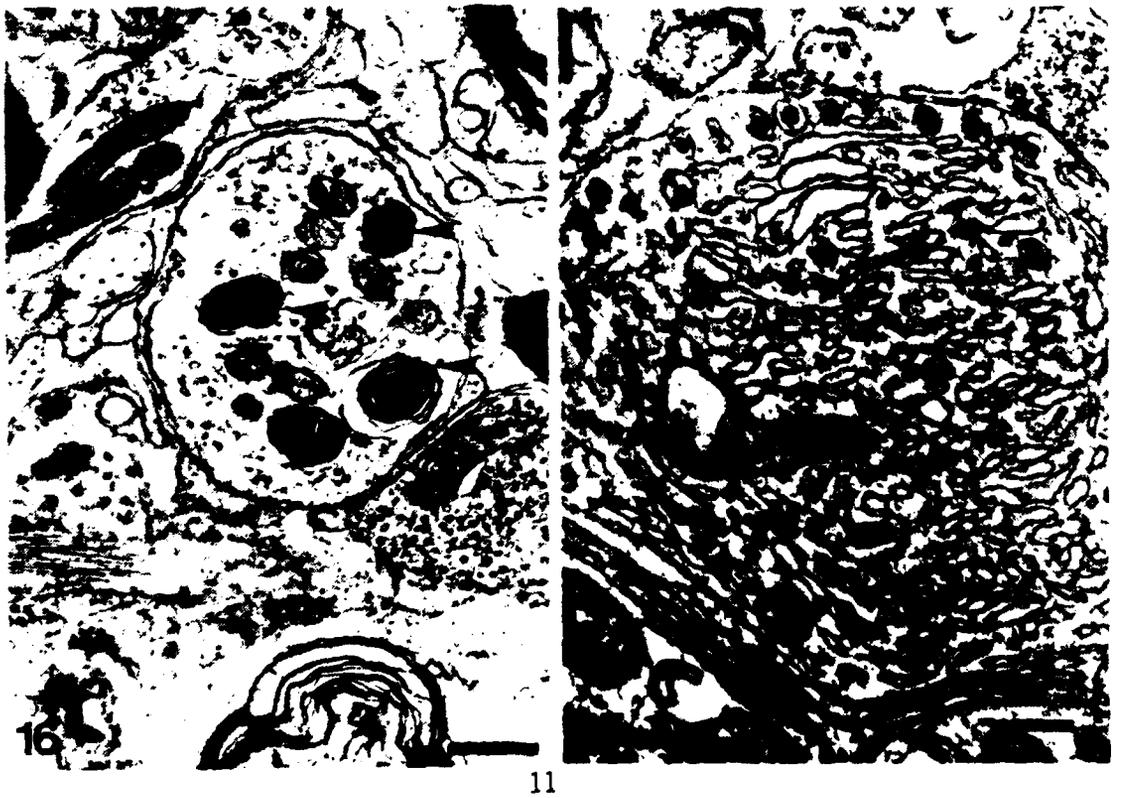


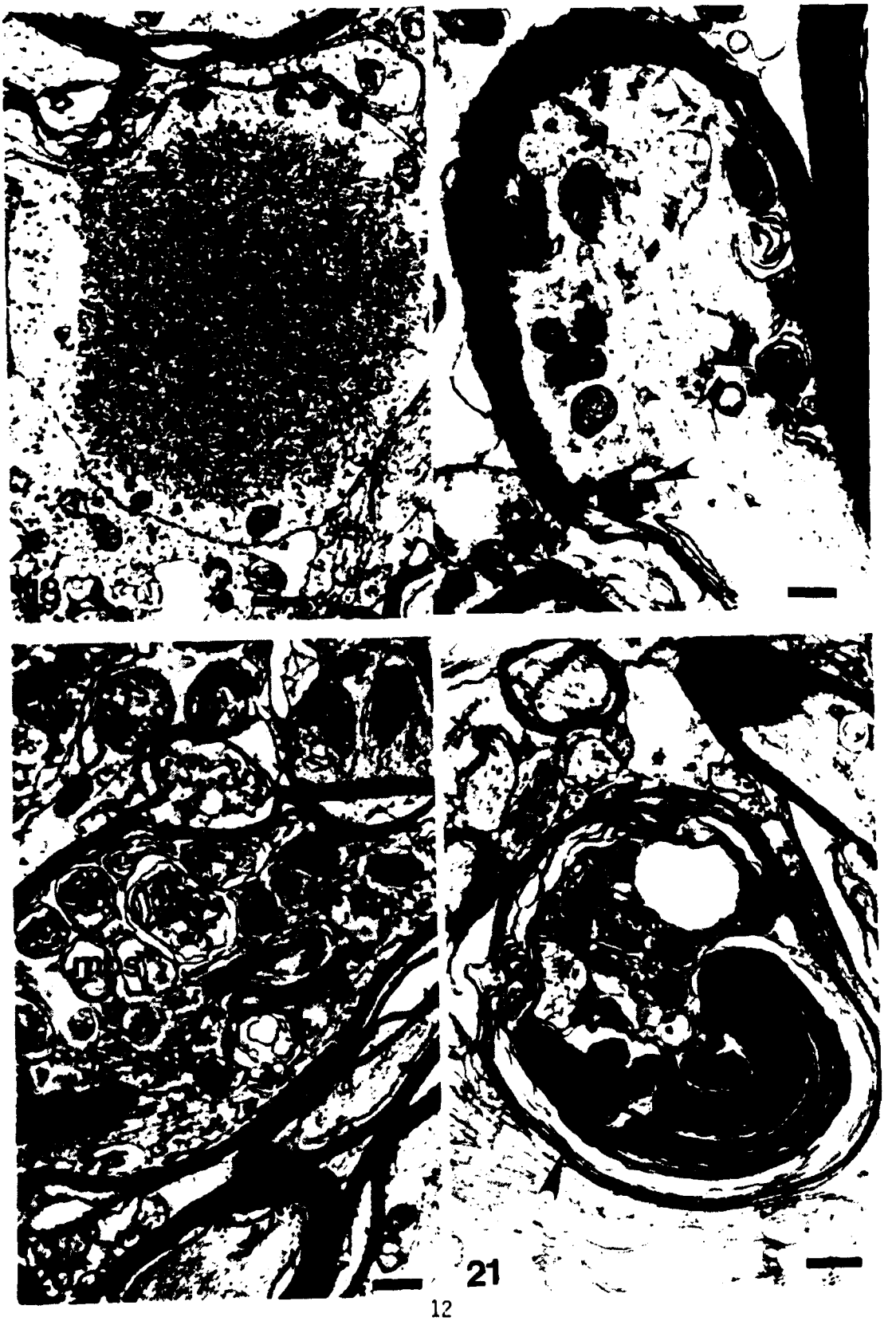
9


12


13
14


16


16



21

B. ELECTROPHYSIOLOGY/NEUROPHARMACOLOGY

The administration of DFP in amounts of 2.0 mg/Kg i.v. in cats pretreated with atropine methyl nitrate (0.3 mg/Kg i.v.) for protection of the peripheral systems in particular the cardiovascular reflexes shows little to no effects on the response of Renshaw cells to 1 or 2 Hz supramaximal antidromic stimulation of L-7 ventral spinal roots during 25 minutes after the injection (fig. 22a-f). However, the response during 20 Hz supramaximal antidromic stimulation (fig. 22g-i) results in a rapid decrease in frequency with absence of response appearing as rapidly as 15 seconds after onset of the repetitive stimulation. Recovery of response after 30 seconds of 20 Hz stimulation (fig. 22j-l), shows a brief tetanic depression (PTD) preceding post tetanic potentiation (PTP) the duration and the frequency of the latter appeared to be greater than that seen in the untreated cat (cf. VanMeter (1983) Annual Report Nr. 1 Figure 13.(a-l)).

Mecamylamine doses of 1.0 mg/Kg i.v. in the presence of atropine methyl nitrate (0.3 mg/Kg i.v. as above) unlike DFP, shortens the duration of the discharge of the Renshaw cells to 1 or 2 Hz supramaximal antidromic ventral root stimulation (fig. 23a-f), with less effect on frequency of the discharge. As seen in figure 23 (a,b,c, cf. d,e,f, respectively) the effect of the drug is rapid, and the burst discharge is reduced to two or three spikes immediately after the stimulus within two to four minutes after injection. Excess endogenous ACh from repetitive stimulation (20 Hz) fails to reverse the antinicotinic action of mecamylamine (fig. 23i) as does persistence of ACh as a result of DFP (fig. 23j and 23k). Also, prolonged higher frequency stimulation (2KHz) fails to alter the action of mecamylamine on the response to Renshaw cells (fig 23l).

Chronic exposure to DFP in sub LD50 doses (0.2mg/Kg/day s.c.) for 14 days (total dose=2.8 mg/Kg s.c.) has little to no effect on Renshaw cell burst response to 1 or 2 Hz stimulation as above (Figures 24 and 25). However, PTP is evoked after 20 Hz supramaximal antidromic stimulation with PTD (fig. 25g-i and 25j-l) or with a rapid recovery to repetitive stimulation followed by PTP (fig. 24g-l).

As with the organophosphate DFP, reversible inhibition of cholinesterases with the carbamate eserine (200 mcgm/Kg i.v.) has little to no effect on Renshaw cell burst responses to 1 or 2 Hz supramaximal antidromic ventral root stimulation (fig. 26a-d), during the peak course of its action (VanMeter and Karczmar 1978). Repetitive antidromic stimulation (20 Hz) (fig. 26e-g), results in a depression of response during the stimulation but a rapid recovery post 20 Hz stimulation during 1 Hz stimulus (fig. 26h-l) with moderate PTP (fig. 26j-l). On the other hand, an increase in the dose of eserine to 500 mcgm/Kg i.v. in the presence of atropine (1.0 mg/Kg i.v.) does antagonise the response of the Renshaw cell to 1 Hz stimulation (fig 27). In Figure 27, atropine fails to alter the Renshaw cell response to 1 Hz antidromic stimulation (fig. 26a and 26b) and a much larger dose of eserine (500 mcgm/Kg i.v.) results in markedly depressed burst responses which are not antagonised by atropine (fig. 27d-f).

RFP are antagonised by 20 Hz antidromic supramaximal ventral spinal root stimulation as seen in Figure 28 (read left to right, and top to bottom). During 'Control,' the pre 20 Hz stimulus (2 Hz) is markedly depressed in both frequency and duration at 30 seconds of 20 Hz

stimulation. Control responses to 20 Hz stimulation are inhibited after 30 to 60 seconds of the repetitive (20 Hz) stimulation. For the purposes of comparison, in regard to time, observations are shown at 30 seconds of 20 Hz stimulation and immediately (0 seconds), 30, and 60 seconds after cessation of 20 Hz and on resumption of 2 Hz antidromic stimulation. Immediately after 20 Hz stimulation the number and frequency of RFP were reduced from prestimulus values 30-60% and 11-15% respectively and recovery of response to pre 20 Hz stimulus values occurs within 3-5 minutes. Eserine (50 mcgm/Kg i.v.) enhances responses to 20 Hz stimulation with inhibition of responses occuring in 10-20 seconds compared to control values of 30-60 seconds. In addition, recovery from 20 Hz stimulation is not observed up to 3 minutes after cessation of 20 Hz stimulation and RFP frequency to 2 Hz stimulation during this time is reduced 18-25% of prestimulus values. While Eserine has no effect on RFP frequency and duration evoked by 2 Hz stimulation during prestimulus control and drug observations, responses to 20 Hz stimulus and recovery are affected in a dose dependent manner (Figure 28 cf Figure 29).

Although Eserine has little to no effect on the RFP during 2 Hz stimulation (cf figs 28,29, and 30.) atropine (0.5 mg/Kg i.v.) given in the presence of Eserine (100 mcgm/Kg i.v.) inhibits RFP 30-40% during 2 Hz stimulation as seen in Fig. 30. In addition mecamylamine (1.0 mg/Kg i.v.) administered in the presence of atropine (30 minutes after) shows further inhibition with only the first two spikes responding to 2 Hz antidromic stimulation. This response to mecamylamine is noted in the absence of eserine and atropine or other drugs as well, and its effects have been observed to persist 30 minutes after administration.

Amphetamine (0.5-1.0 mg/Kg i.v.) fails to show effects on either 2 Hz or 20 Hz antidromic stimulation (Figs. 31 and 32). Moreover, a sub effective does of Eserine (20 mcgm/Kg i.v.) determined in preliminary experiments is not potentiated by amphetamine (Fig 32).

RENSHAW CELL RESPONSE TO REPETITIVE STIMULATION AFTER ACUTE DFP

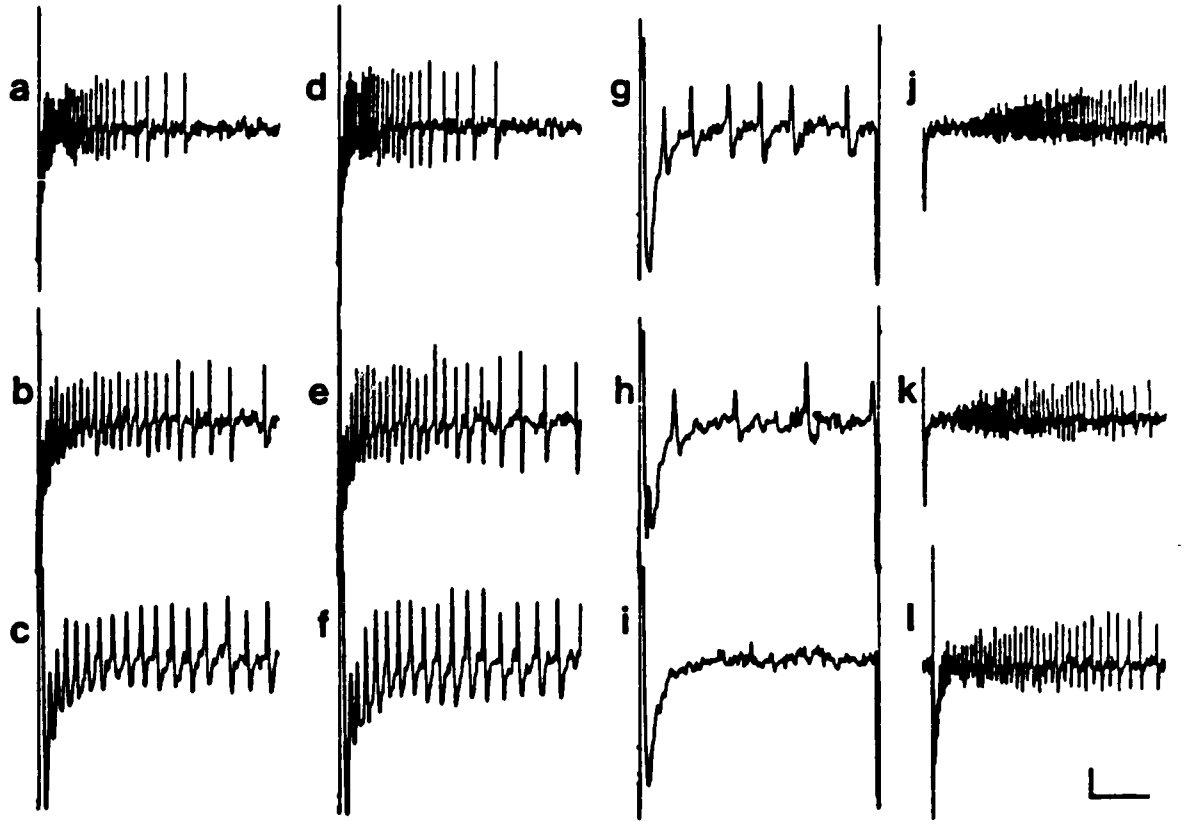


Figure 22. Renshaw cell response to 20 Hz antidromic L-7 ventral root stimulation in the presence of 2.0 mg/Kg i.v. DFP and atropine methyl nitrate 0.3 mg/Kg i.v.. a. Control response to 1 Hz antidromic stimulation in the presence of atropine methyl nitrate 0.3 mg/Kg i.v.. b.& c. Same at increasing CRO sweep rates. d. Response to 1 Hz antidromic stimulation as before but 15 minutes after DFP 0.5 mg/Kg i.v.(total dose = 2.0 mg/Kg i.v.). e.& f. Same at increasing CRO sweep rates. g.,h.,& i. Response after 5,10,& 15 secs. of 20 Hz antidromic stimulation, respectively. j.,k.,& l. Response to 1 Hz antidromic stimulation 5,30,& 120 secs.after 30 secs.of 20 Hz antidromic stimulation. Calibration signal: 50 μV/15 msec (c,f,g,h,& i), 50 μV/25 msec (b.& e.), 50 μV/50 msec (a.& d.), 100 μV/50 msec (l.), 100 μV/125 msec (j.& k).

MECAMYLAMINE ANTAGONISM OF RENSHAW CELL DISCHARGE AND DFP

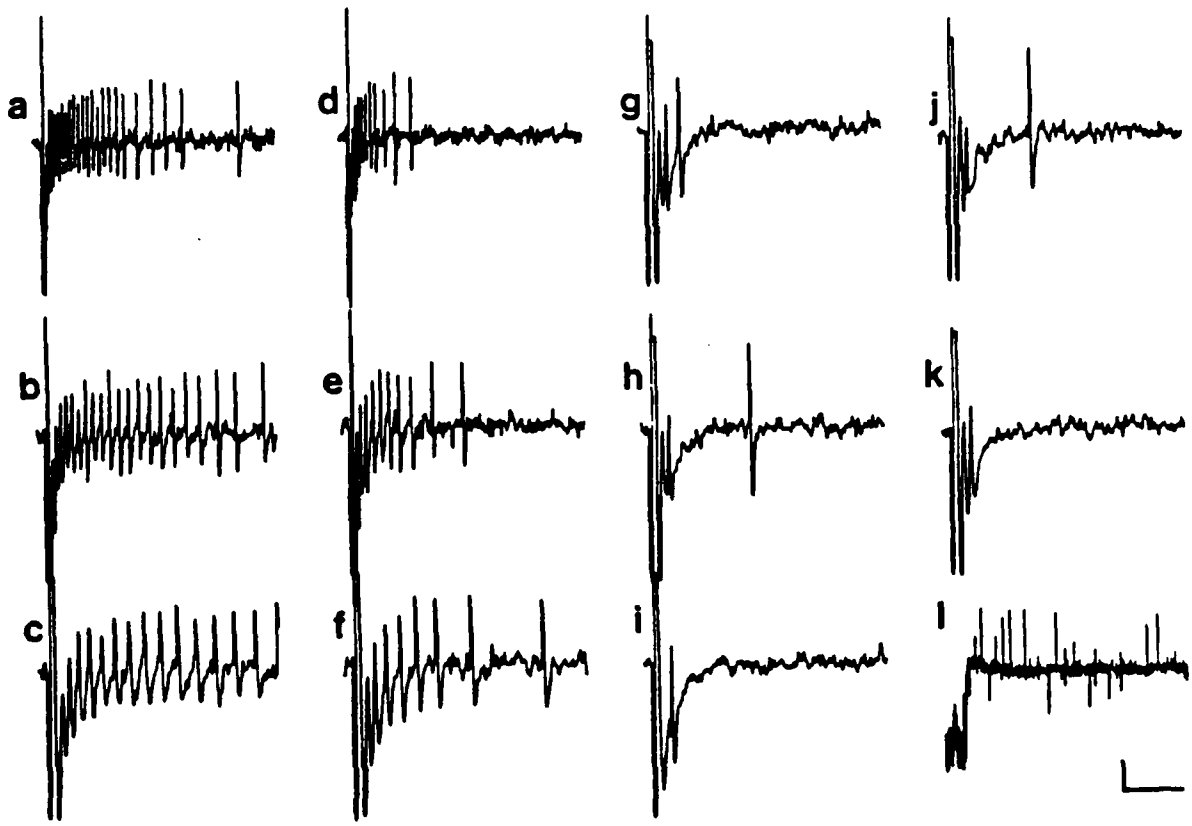


Figure 23. Mecamylamine antagonism of Renshaw cell discharge is not reversed by DFP. a.Control response of Renshaw cell to 1 Hz antidromic L-7 ventral root supramaximal stimulation. b.& c. Same response at increasing CRO sweep rates. d. Response 1 min. after mecamylamine 1.0 mg/Kg i.v.. e.& f.Same responses at increasing CRO sweep rates. g.& h.Response after 2 and 4 minutes respectively. i.Response to 1 Hz stimulation after 30 secs. of 20 Hz L-7 antidromic ventral root stimulation. j.Response to 1 Hz stimulation 7 min after DFP 2.5 mg/Kg i.v. k.Response to 1 Hz stimulation after 3 min of 20 Hz antidromic L-7 ventral root stimulation. l. Asynchronous discharge evoked by 2 KHz antidromic ventral root stimulation (lower left at the beginning of the trace). Calibration Signal: 50 μV/15 msec (c,f,g,h,i,j,& k). 50 μV/25 msec (b & e), 50 μV/50 msec (a & d) and 50 μV/0.25 secs. (l).

A:RENSHAW CELL RESPONSE TO 20 Hz STIMULATION AFTER CHRONIC DFP

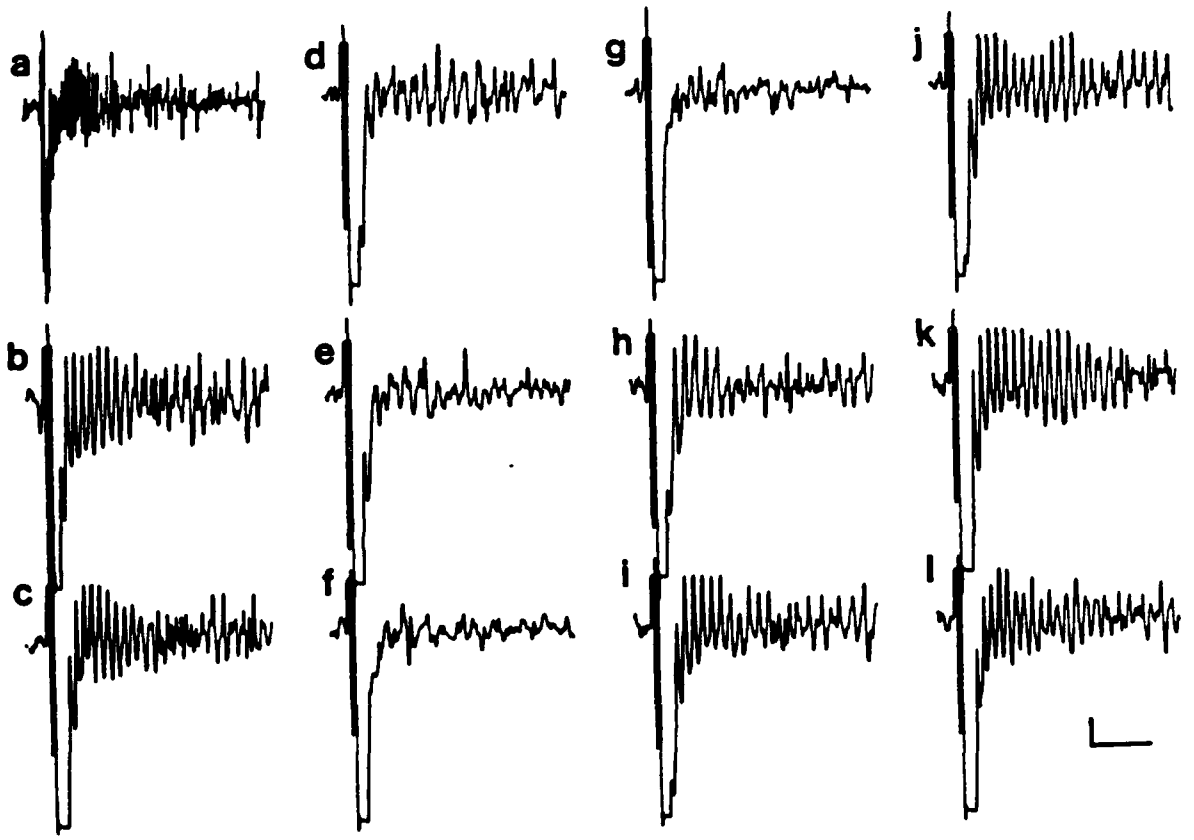


Figure 24. Renshaw cell response from a cat given DFP 0.2 mg/Kg/day for 14 days (total dose = 2.8 mg/Kg s.c.)with 0.3 mg/kg i.v. atropine methyl nitrate on the day of recording shows post tetanic potentiation but not post tetanic depression after 60 secs. of 20 Hz antidromic stimulation. a.Control response of Renshaw cell to 1 Hz antidromic L-7 ventral root supramaximal stimulation. b.& c. Same at increasing CRO sweep rates. d.,e.,f.,& g. After 2,10,30,& 60 secs of 20 Hz antidromic stimulation,respectively. h.,i.,j.,k.,& l. Response to 1 Hz antidromic stimulation, 2,10,30 & 60 secs, & 3 min after 20 Hz antidromic stimulation,respectively.Calibration signal: 50 μV/15 msec (c.- l.), 50 μV/25 msec (b.), and 50 μV/50 msec (a.).

B:RENSHAW CELL RESPONSE TO 20 Hz STIMULATION AFTER CHRONIC DFP

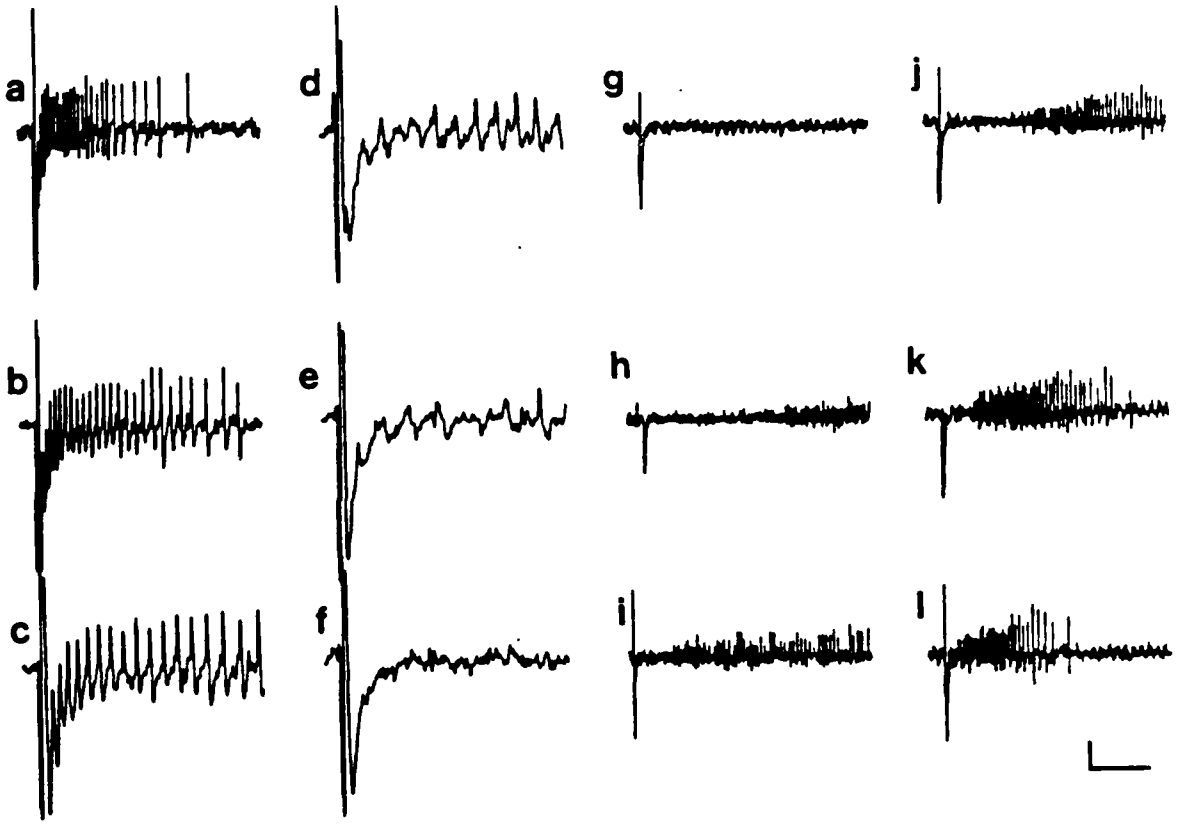


Figure25. Renshaw cell response from a cat given DFP 0.2 mg/Kg/day for 14 days (total dose = 2.8 mg/Kg s.c.) with 0.3 mg/Kg i.v. atropine methyl nitrate on the day of recording, shows post tetanic depression and potentiation after 60 secs. of 20 Hz antidromic stimulation. a. Control response of the Renshaw cell to 1 Hz antidromic L-7 ventral root supramaximal stimulation. b.,& c. Same at increasing CRO sweep rates. d.,e.,& f. After 2,30, and 60 secs of 20 Hz antidromic stimulation, respectively. g.- l. Recovery of response to 1 Hz antidromic stimulation 5,10,15,30,60 & 120 secs. after 20 Hz antidromic stimulation. Calibration signal: 50 μV/15 msec (c-f), 50 μV/25 msec (b), 50 μV/50 msec (a), 100 μV/125 msec (g.,j.,k.,&l.), 100 μV/250 msec (h.& i.).

RENSHAW CELL RESPONSE TO REPETITIVE STIMULATION AFTER ESERINE

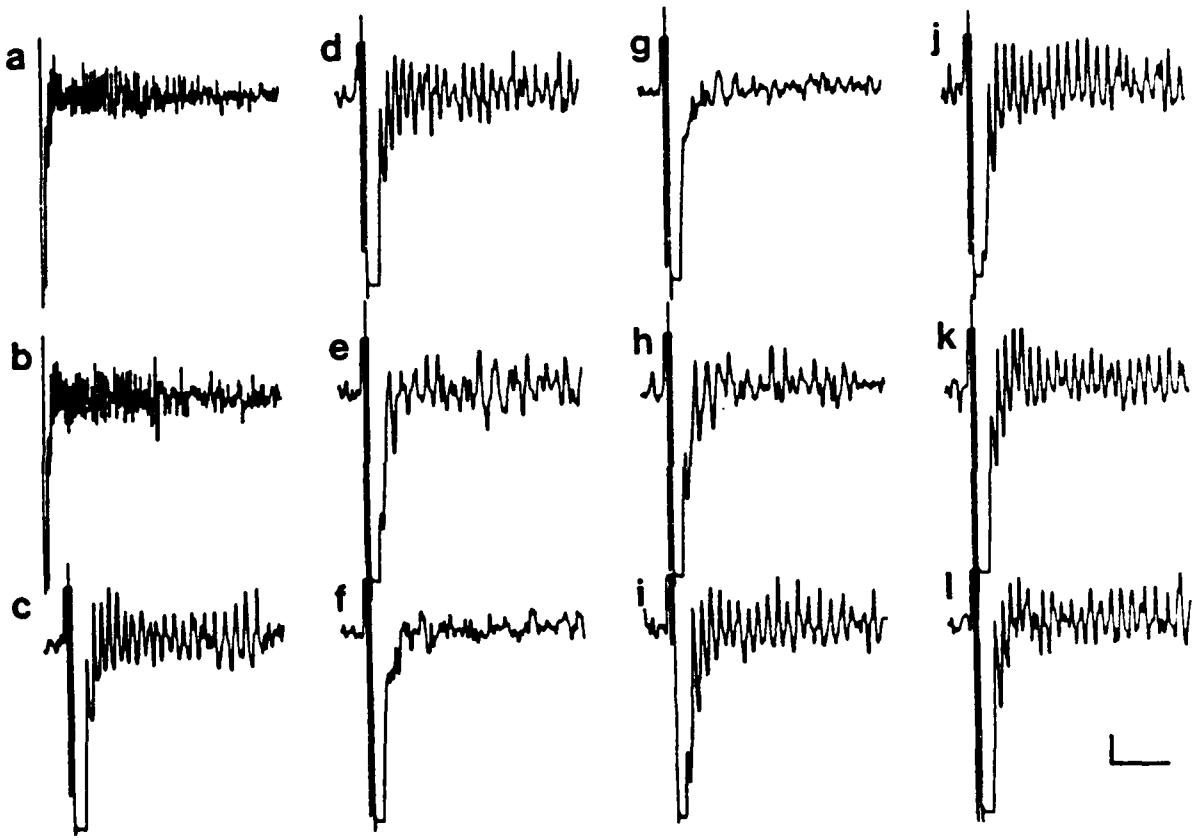


Figure 26. Post tetanic potentiation of a Renshaw cell after eserine 200 µgm/Kg i.v. in the presence of atropine methyl nitrate 0.3 mg/Kg i.v.. a.Control response of Renshaw cell to 1 Hz antidromic L-7 ventral root stimulation. b.Response to 1 Hz stimulation 5 min. after eserine 200 µgm/Kg i.v. c.Same but at a faster CRO sweep rate. d. Response to 1 Hz stimulation 9 minutes after eserine and just prior to 20 Hz antidromic stimulation. e.,f.,& g. After 2,30,& 60 secs. of 20 Hz antidromic stimulation. h.-l. Response to 1 Hz antidromic stimulation at 2,15,30,& 60 secs. and 3 min. respectively. Calibration signal: 50 µV/15 msec (c.-l.), 100 µV/50 msec (a.& b.).

RENSHAW CELL DISCHARGE AFTER ESERINE IN PRESENCE OF ATROPINE

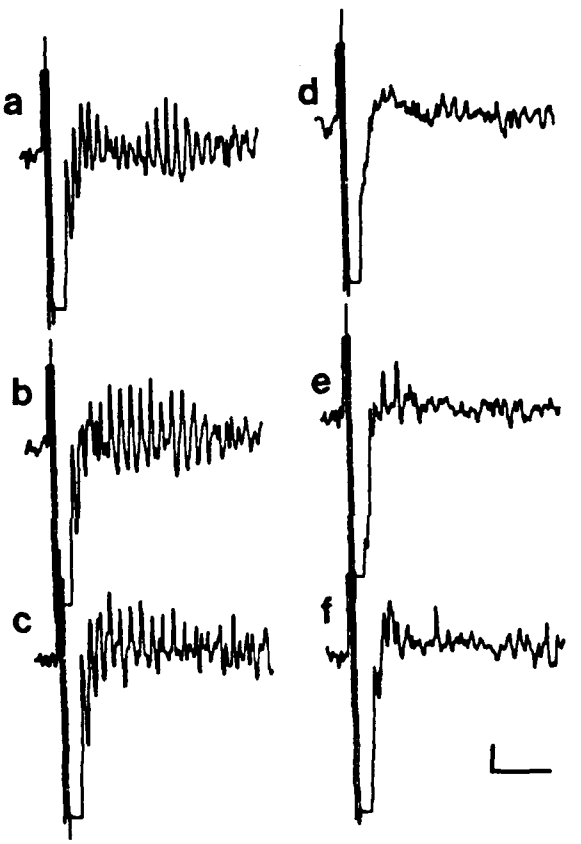


Figure 27 Atropine sulfate (1.0 mg/Kg i.v.) fails to protect against eserine antagonism of Renshaw cell discharge. a. Control response of Renshaw cell to 1 Hz antidromic L-7 ventral root stimulation. b. Response at 5 min after atropine sulfate (1.0 mg/Kg i.v.). c. Response on injection of 500 µgm/Kg i.v. eserine. d., e., & f. Response 1,5,& 10 min after eserine. Calibration signal: 50 µV/15 msec.

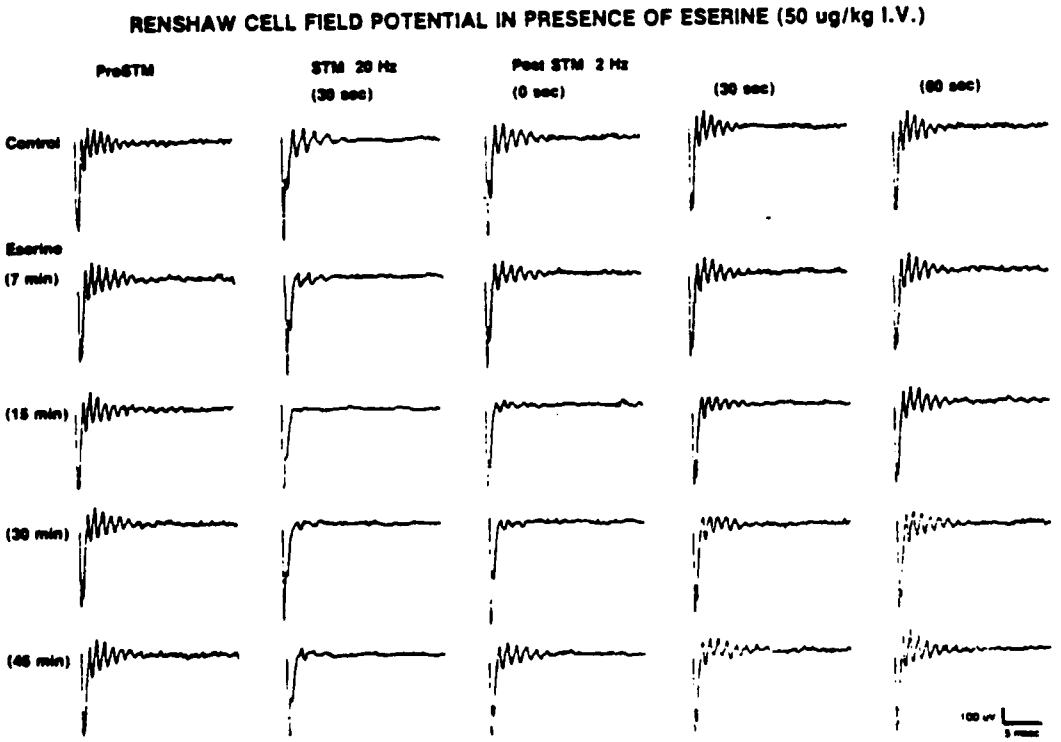


Figure 28 PreSTM 2 Hz: Response to 2 Hz antidromic supramaximal L-7 ventral spinal root stimulation during 'Control' and 7,15,30,and 45 minutes after Eserine (50 µgm/Kg i.v.).STM 20 Hz (30 sec): After 30 seconds of 20 Hz antidromic supramaximal L-7 ventral spinal root stimulation and 7,15,30,and 45 minutes after Eserine (50 µgm/Kg i.v.). Post STM 2 Hz (0 sec),(30 sec), and (60 sec): Response to 2 Hz antidromic stimulation as above 0,30,and 60 seconds respectively,after 30 seconds of 20 Hz antidromic stimulation during 'Control' and 7,15,30, and 45 minutes after Eserine (50 µgm/Kg i.v.).

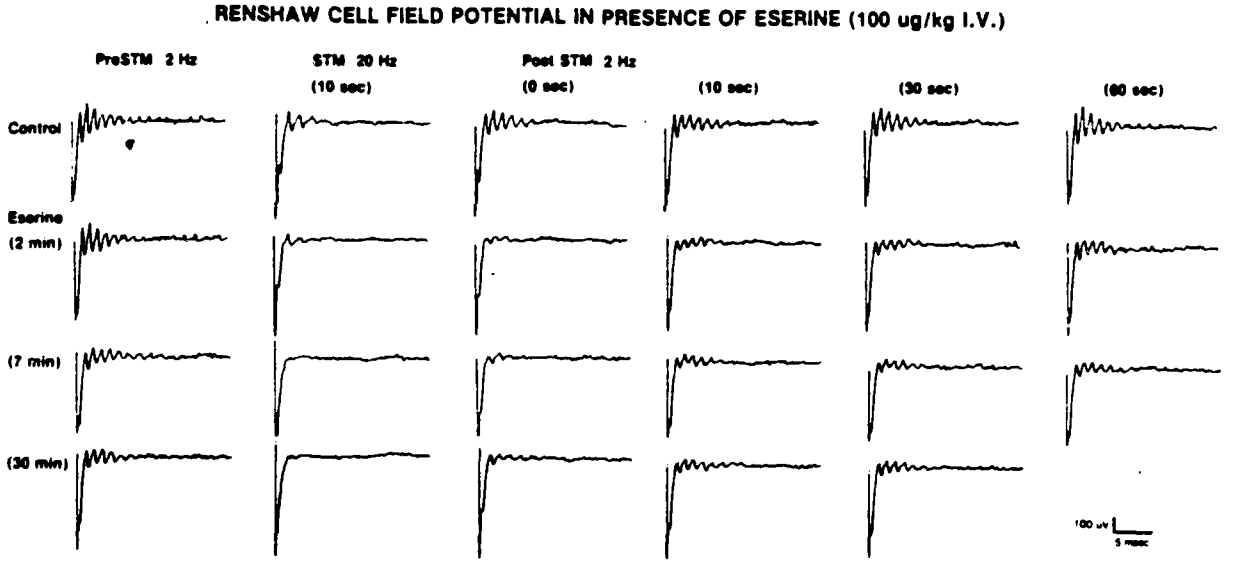


Figure27. PreSTM: Response to 2 Hz antidromic supramaximal L-7 ventral spinal root stimulation during 'Control' and 2,7,and 30 minutes after Eserine (100 µgm/Kg i.v.). STM 20 Hz (10 sec): Response after 10 seconds of 20 Hz antidromic supramaximal L-7 ventral spinal root stimulation and after 2,7, and 30 minutes Eserine (100 µgm/Kg i.v.). Post STM 2 Hz (0 sec),(10 sec),(30 sec), and 60(sec): Response to 2 Hz antidromic stimulation as above 0,10,30,and 60 seconds respectively,after 10 seconds of 20 Hz antidromic stimulation as seen during 'Control' and 2,7 and 30 minutes after Eserine (100 µgm/Kg i.v.).

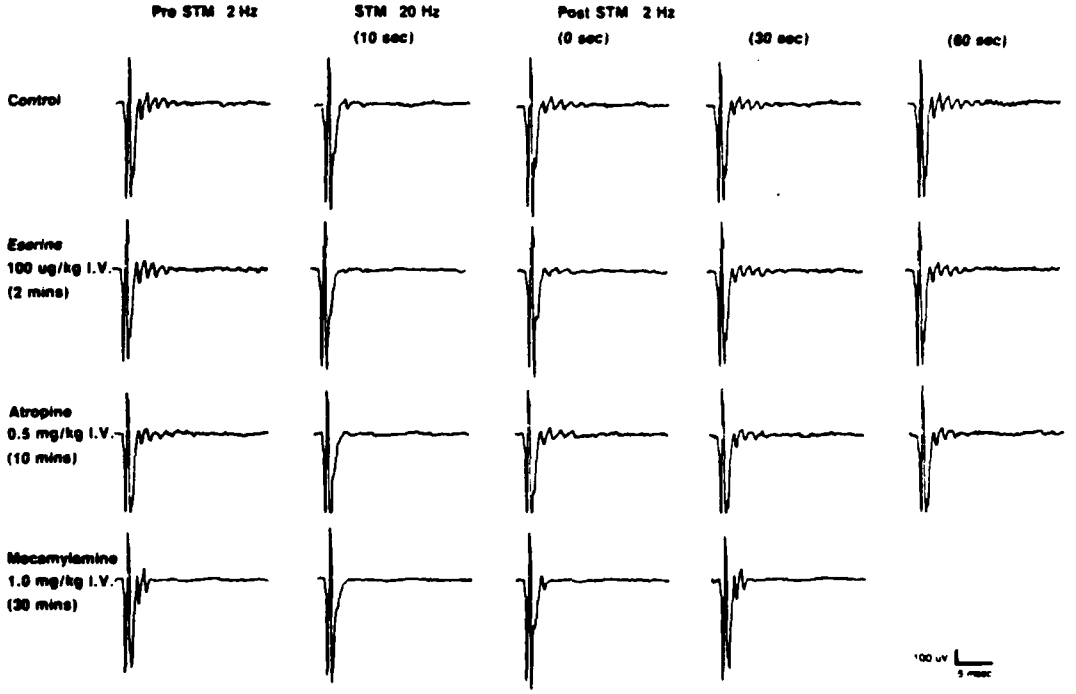


Figure 28. PreSTM 2 Hz: Response to 2 Hz antidromic supramaximal L-7 ventral spinal root stimulation during 'Control' and 2 minutes after Eserine (100 µgm/Kg i.v.), 10 minutes after atropine (0.5 mg/Kg i.v.) and 30 minutes after mecamylamine (1.0 mg/Kg i.v.). STM 20 Hz(10 sec): Response after 10 seconds of 20 Hz antidromic supramaximal L-7 ventral root stimulation and 2 minutes after Eserine (100 µgm/Kg i.v.), 10 minutes after atropine (0.5 mg/Kg i.v.), and 30 minutes after mecamylamine(1.0 mg/Kg i.v.). Post STM 2 Hz (0 sec),(30 sec), and (60 sec) : Response to 2 Hz antidromic stimulation as above 0,30 and 60 seconds after 10 seconds of 20 Hz antidromic stimulation as seen during 'Control' and 2 minutes after Eserine (100 µgm/Kg i.v.), 10 minutes after atropine (0.5 mg/Kg i.v.) and 30 minutes after mecamylamine (1.0 mg/Kg i.v.).

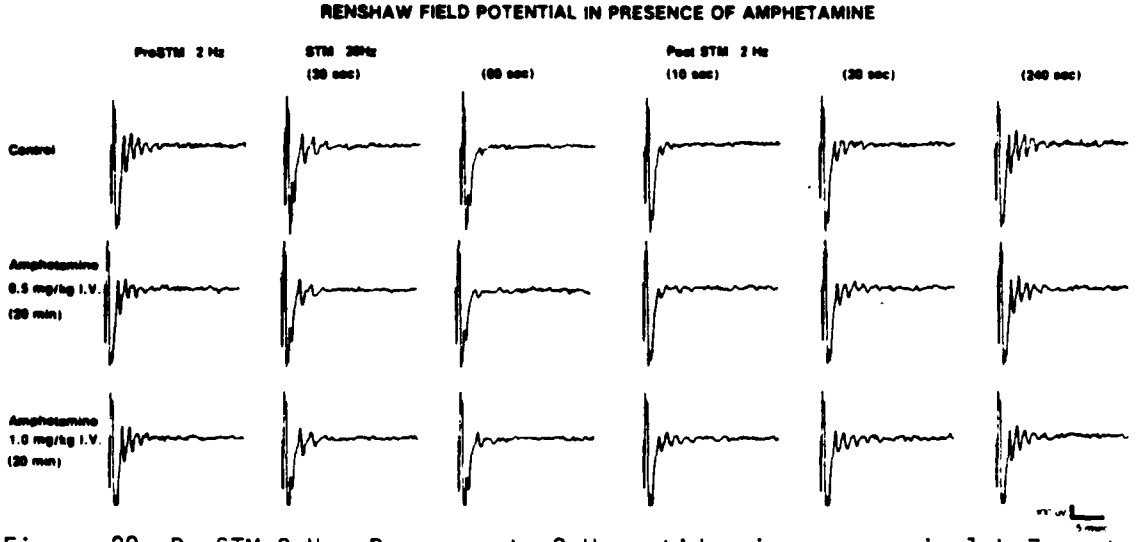


Figure 29. PreSTM 2 Hz: Response to 2 Hz antidromic supramaximal L-7 ventral spinal root stimulation during 'Control' and 20 minutes after amphetamine 0.5 mg/Kg i.v., and 20 minutes after amphetamine 1.0 mg/Kg i.v.. STM 20 Hz (30 sec),(60 sec): Response after 30 and after 60 seconds of 20 Hz antidromic supramaximal ventral root stimulation respectively, and 20 minutes after amphetamine (0.5 mg/Kg i.v.) and 20 minutes after amphetamine (1.0 mg/Kg i.v.). Post STM 2 Hz (10 sec),(30 sec) and(240 sec) : Response to 2 Hz antidromic stimulation as above 10, 30, and 240 seconds after 20 Hz antidromic stimulation as seen during 'Control'and 20 minutes after amphetamine (0.5 mg/Kg i.v.) and 20 minutes after amphetamine (1.0 mg/Kg i.v.).

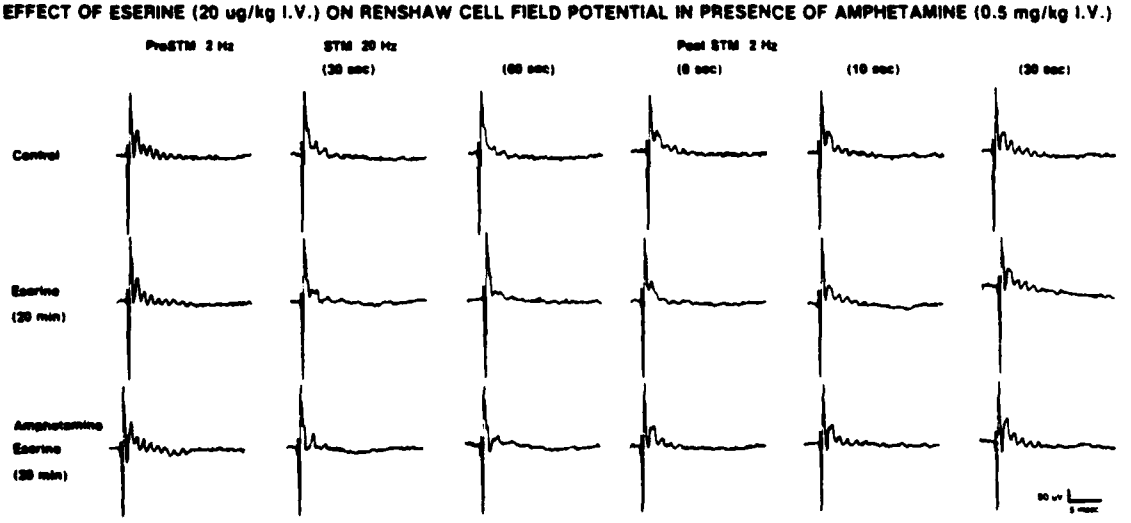


Figure 30. PreSTM 2 Hz: Response to 2 Hz antidromic supramaximal L-7 ventral spinal root stimulation during 'Control' and 20 minutes after Eserine (20 µgm/Kg i.v.) and 20 minutes after Eserine (20 µgm/Kg i.v.) in the presence of Amphetamine (0.5 mg/Kg i.v.). STM 20 Hz (30 sec), (60 sec). Response after 30 and after 60 seconds of 20 Hz antidromic supramaximal ventral root stimulation respectively, and after Eserine,and Amphetamine and Eserine as above. Post STM 2 Hz (0 sec), (10 sec), and (30 sec): Response to 2 Hz antidromic stimulation as above 0,10,and 30 seconds after 20 Hz antidromic stimulation as seen during 'Control' and after Eserine and Amphetamine and Eserine as above.

DISCUSSION

A. MORPHOLOGY

Acute toxic effects result from the administration of many organophosphates known to inhibit the enzyme acetylcholinesterase. In addition, some organophosporus esters produce a delayed neurotoxic effect which may be initiated by inhibition of a neurotoxic esterase (Johnson (1975)) and histopathologic lesions in the central nervous system occur with delayed neurotoxicity after intoxication with DFP (Barnes and Denz (1953) Bouldin and Cavanagh (1979), Bouldin and Cavanagh (1979a), Cavanagh (1954), and Frenton (1955)) and TOCP (Barnes and Denz (1953), Bischoff (1970), Cavanagh (1954), Abou-Donia (1978), for review see Abou-Donia (1981)). Initial structural changes are observed in the spinal tracts twelve days after exposure to DFP or TOCP and are more marked after 21 days (Cavanagh (1954), Cavanagh (1965), and Frenton (1955)). Clinical characteristics of delayed neurotoxicity are weakness and ataxia in the lower limbs, progressing to paralysis with the upper extremeties becoming involved in more severe cases. According to Johnson (1975) symptoms of delayed neurotoxicity are not seen until 8 to 14 days after exposure to the organophosphate. Even if daily doses are given, signs of ataxia do not appear until the eighth day and Bouldin (1979 and 1979a) observed hind-limb ataxia in cats at day 16 to 18 after DFP. With daily injections of DFP the onset of ataxia in the animals used for our studies was dose dependent and varied from day 4 to day 12. It has been previously shown that delayed neurotoxic effects of some organophosphates have a dose-dependent response and are related to frequency and duration of exposure (for review see Abou-Donia (1981)). Also, subneurotoxic doses given at varying time intervals accumulate to a dose that causes delayed neurotoxicity (Cavanagh (1964), Cavanagh (1964a), and Davies (1963), for review see Abou-Donia (1981)).

The present study shows chronic subcutaneous administration of DFP to cumulative amounts less than the LD50 (1.4 mg/kg) or greater than the LD50 (10.5 mg/kg) from 5 to 21 days results in morphological evidence of neurotoxicity. Moreover, the earliest a chronic treated animal was killed was after 5 days treatment with 0.75 mg/kg DFP (cumulative dose 3.75 mg/kg) and therefore it is unclear whether or not this neurotoxicity is "delayed." Delayed neurotoxicity, however, cannot be excluded since the findings are dose dependent and probably occur earlier than previously described (Bouldin (1979), Bouldin (1979a), and Johnson (1975)). On the other hand, a subacute effect of DFP cold be responsible for the morphological changes in the earlier stages of the treatment with the additional occurrence of delayed neurotoxicity after longer periods of time. In contrast, morphological neurotoxicity is not seen in tissue taken 1 to 17 hours after a single low dose intravenous injection of DFP (0.1 mg/kg or 0.2 mg/kg). Moreover, Glazer et al. (1978), observed subacute as well as delayed neurotoxic effects in the cat soleus nerve-muscle system after a single much greater injection of DFP (2 mg/kg). The acute effects (widened primary clefts, loss of junctional folds, etc.) occur one to three days after this administration of DFP followed by recovery and finally degeneration of motor nerve terminals as a result of delayed neurotoxicity. The same events may take place in the spinal cord of cats. However, our

animals received daily treatment which precludes recovery, and additional studies are needed to determine the relationship between fine structural evidence for neurotoxicity resulting from acute exposure and that from chronic exposure.

Histopathological changes in the central nervous system due to the neurotoxic effect of organophosphates are described to be most evident in the anterior columns of thoracic and lumbar spinal cord (Abou-Donia (1978a), Abou-Donia and Graham (1978), Abou-Donia et al. (1980), Ahmed (1971), Cavanagh (1965), and Gless and Janzik (1965)). Changes mostly involve nerve fibers and terminals. Other authors report no change in the nerve cell bodies that could account for the extensive damage to the nerve fibers (Abou-Donia (1981), Bouldin (1979a), Cavanagh (1954), Cavanagh (1964)). Some pathological alterations of the neurones which occur mainly in the perikaryon are discribed by Bischoff (Bischoff (1970)) four days after the onset of clinical signs in chickens with TOCP administration. Our studies are in general agreement, since acute exposure to DFP does not change motoneurone cell bodies. However, slight changes such as an increase of lysosomal elements in many motoneurones could be observed after the chronic administration. It has been suggested in previous studies that lysosomal enzymes play an important role in the development of nerve degeneration (Abou-Donia (1978), Abou-Donia (1978a)). If organophosphates inhibit axoplasmic transport (Couraud and DiGiamberardino (1980), Lubinska and Niemierko (1971), and Reichert and Abou-Donia (1980)), accumulation of proteins synthesized in the soma and usually transported down the axon by fast axoplasmic transport could result in increased lysosomal activity. However at present, the effect of organophosphorus compounds on axoplasmic transport remains controversial (Abou-Donia (1981), Bradley and Williams (1973), James and Austin (1970), and Pleasure et al. (1969)).

In the ventral horn of the cat and chicken spinal cord, synaptic degeneration is described two to three weeks after a single dose of TOCP (Bischoff (1970) and Illis et al. (1966)). The number of degenerating synapses is large and shows varying degrees of severity (Ahmed (1971)). Our studies reveal early signs of degeneration in the presynaptic terminals with 0.1 mg/kg DFP over 14 days, a dose which fails to cause neuropathological signs in the axon.

Besides degenerative processes in the terminals, which occur after chronic DFP exposure, there are increased amounts of coated vesicles. The increase appears after a single exposure to DFP as well as after chronic treatment. Furthermore, the coated vesicles are present in the preterminal portions of many axons. According to Heuser and Reese (1973) coated vesicles are part of the "membrane recycling" process. After continuous stimulation of frog nerve-muscle preparations these authors report a decrease in synaptic vesicles and an increase in coated vesicles. They conclude that coated vesicles play an essential role in the presynaptic recovery process after stimulation. Moreover, Laskowski et al. (1977) find an accumulation of coated vesicles in the nerve terminals of motor endplates after paraoxon exposure. The increase in coated vesicles begins when transmitter release is high (30 minutes to 6 hours after paraoxon). These authors propose excessive vesicle turnover to be responsible. Furthermore, they describe a progressive increase in coated vesicles over the next 24 to 72 hours, which they suggest is due to a block in the

recycling process. The increased amount of coated vesicles in the presynaptic terminals in our cats may be dose-dependent and quantitative studies are being undertaken. Preliminary studies (Sikora-VanMeter et al. unpublished observations) show that animals treated chronically with DFP and allowed to recover for 3 days contain an even greater number of coated vesicles in the presynaptic terminals than animals which were not allowed to recover from the chronic treatment. This increase in coated vesicles could be a delayed effect of DFP which is in support of the studies of Laskowski et al. (1977) discussed above.

In addition, some organophospates are known to produce "dying back" neuropathy, where axonal degeneration primarily appears in the distal ends of the longest and largest fibers while the proximal portions and their perikarya remain intact (Cavanagh (1964) and Prineas (1969)). Bouldin et al. (1979 and 1979a) report that a single injection of DFP induces axonal degeneration after 18 and 20 days which does not start at the terminal but spreads subsequently in a somatofugal direction and involves the entire distal axon. The authors conclude that organophosphates induce a focal, distal, but not terminal axon degeneration. In our studies degeneration is observed in non-myelinated portions of nerve fibers (either preterminal portions of myelinated axons or non-myelinated fibers) before changes in myelinated ones occur. Extensive damage to myelinated axons occurs in animals treated either with low doses of DFP over extended periods of time or with high but sub LD50 doses over shorter periods of time. The degree of morphological damage is dependent upon dose and duration of treatement. Demyelination occurs if the axon itself shows degenerative changes. Furthermore, our studies indicate that demyelination in DFP treated animals might be a secondary effect due to axon degeneration as suggested by Cavanagh (1954). Ahmed and Gless (1968) find early axonal changes in the ventromedial tract of the lumbar spinal cord of hens after exposure to TCP. The axons eventually disappear completely and degenerating myelin fills the space formerly occupied by the axon. The authors suggest that full flow of axoplasm is necessary to maintain a normal myelin sheath.

The present study of fine structural details associated with the early morphological neurotoxicity after chronic sub LD50 exposure to DFP shows degeneration and demyelination of axons in the ventral portion of the ventral horn to be dependent on dose and the duration of treatment. Furthermore, the DFP-induced axonal degeneration is likely to be a retrograde process which begins at the presynaptic terminal.

B. ELECTROPHYSIOLOGY/NEUROPHARMACOLOGY

The response of Renshaw cells to systemically applied antiChE's that can penetrate the blood brain barrier (BBB) and to the microiontophoretical application of these centrally acting as well as others which do not cross the BBB, consists of an increase in discharge frequency and duration (Eccles et al. (1954)). These responses are dependent on experimental conditions to include anaesthetic used (Biscoe and Krnjevic (1963)), the parameters of the antidromic stimulation (Ross et al. (1972)) and the dose (Eccles et al. (1954), Eccles et al. (1956), Longo et al (1960), and Curtis et al. (1961)). In general, our results support the findings in the literature and while the results shown in Figure 22a-1f

are not as dramatic as those cited, the anaesthetic used and the dosage of antiChE may be accountable. In addition, the response to 20 Hz antidromic stimulation shows a rapid decline in frequency to a point where response to 20 Hz stimulus fails. The pathway for antidromic activation of Renshaw cells is cholinergic monosynaptic transmission via axon collaterals from the motoneurone fibers being stimulated (Eccles et al. (1954), Eccles (1964)). The Renshaw cells when sufficiently activated fire a burst discharge consistent with its excitatory post synaptic potential (EPSP) and the burst, when transmitted, initiates inhibitory recurrent post synaptic potentials (RIPSP) in membranes of motoneurones and other interneurones (Eccles (1964), Hultborn et al. (1971)). The source for activation of the Renshaw cells, i.e. terminals on the motoneurone axon collaterals, is thus being inhibited and the decrease of Renshaw cell firing rate to point of failure during the 20 Hz antidromic stimulation may reflect an increased recurrent inhibition of the motoneurone. In addition, other factors such as "mutual inhibition" (Renshaw (1946), Ryall (1970), and Ryall et al. (1971)) may also contribute. However, since the decrease in frequency and antagonism of response is more evident in the presence of DFP either given acutely (Figure 22) or chronically (Fig. 24 & 25), this would suggest a cholinergic facilitation of synaptic transmission resulting ultimately in a more efficient recurrent inhibition. Also during the recovery from 20 Hz stimulation the PTP appears enhanced from that seen in non-treated cats (cf Annual Report 1). The delay in response during the post 20 Hz stimulus (PTD) may reflect Renshaw cell recovery from this increased efficiency of recurrent inhibition induced by repetitive stimulation. However, Krnjevic and Lekic (1977) reported a PTD of variable duration after tetanic stimulation and further that microiontophoretically applied ACh and aspartate were less effective during PTD, which would imply a non-specific mechanism. Also, since Renshaw cells have been demonstrated to be affected by other agonists and antagonists, additional mechanisms may be involved (Curtis et al. (1976), Belcher and Ryall (1977), and Davies and Dray (1976)). In any event, a more complete analysis of these data by computer and additional experiments with microiontophoretic application of ACh and putative transmitters as well as antagonists are in progress.

Mecamylamine, a centrally acting antinicotinic drug, effectively antagonised the response of Renshaw cells to 1 or 2 Hz antidromic stimulation (Figure 23) which is in agreement with literature (Ryall et al. (1971)). In addition, the effect of mecamylamine was initially on the duration of the discharge and at peak effect, there were characteristically one or two spikes which appeared resistant to the antinicotinic drug (cf Figure 23g and 23h). Attempts to reverse this antagonism either with repetitive stimulation to induce excess ACh or by the administration of DFP to prolong the action of released transmitter failed (cf Figure 23i and 23k, and Figure 23j and 23k, respectively). Thus, mutual antagonism as is seen between atropine and antiChE's such as DFP or sarin (VanMeter and Karczmar (1978)) is not a characteristic of mecamylamine antagonism of Renshaw cell activity.

While Eserine in doses of 100-200 mcgm/Kg i.v. routinely elicits a potentiation of the Renshaw cell response to antidromic stimulation (Figure 26), in higher doses (Figure 27, 500mcgm/Kg i.v.) there is frequently an antagonism of the response (Figure 27d) which is only slightly reversed by

large doses (1.0 mg/Kg i.v.) of atropine (Figure 27e and 27f). On the other hand, Renshaw cell field potentials (RFP) show little to no reaction to Eserine in doses of 50 and 100 mcgm/Kg i.v. during 1 or 2 Hz stimulation but the effect of Eserine is apparent with 20 Hz stimulation as the depression of response is more pronounced, of longer duration, and is dose dependent (Figs. 28 and 29).

Antagonism studies with atropine and mecamylamine show that recovery from repetitive stimulation in the presence of Eserine is enhanced by atropine which by itself has little to no effect on 2 Hz stimulation (Figure 30). On the other hand, mecamylamine antagonises the response to 2 Hz stimulation but not the first two spikes after stimulation in agreement with results from RUP (cf Figure 23). These spikes are antagonised during 20 Hz stimulation. A similar effect was noted for RUP during 20 Hz stimulation (cf Figure 23i).

Anticholinesterases (Eserine, DFP, sarin) have been shown to antagonise evoked potentials in the CNS, in particular thalmocortical recruitment and caudatocortical burst activity. While the adrenomimetic amphetamine, like the centrally acting antiChE's induces a desynchronised EEG, it fails to antagonise the evoked potentials (VanMeter (1977)). However, sub effective doses of Eserine have been shown to be potentiated by the presense of amphetamine and to antagonise thalamocortical recruitment (VanMeter (1977)). Since thalamocortical recruitment may also be mediated through a cholinergic system (VanMeter and Karczmar (1971), VanMeter and Karczmar (1978)), a comparative study was undertaken to determine if the effect of a sub-threshold dose of Eserine on RFP could be enhanced by amphetamine. In Figure 31 amphetamine enhanced the recovery of the RFP to 20 Hz stimulation in a dose dependent manner and this effect persisted in the presence of Eserine (Figure 32). Therefore, these two drugs do not potentiate but rather the action of amphetamine antagonises the effect of Eserine on RFP response to and recovery from 20 Hz stimulation, which suggests a noncholinergic effect on RFP.

LITERATURE CITED:

Abou-Donia, M.B. (1978) Role of Acid Phosphatase in Delayed Neurotoxicity Induced by Leptophos in Hens. Biochem. Pharmacol. 27: 2055-2058.

Abou-Donia, M.B. (1978a) Increased Acid Phosphatase Activity in Hens Following an Oral Dose of Leptophos. Toxicol. Lett. 2: 199-203.

Abou-Donia, M.B. and D.G. Graham (1978) Delayed Neurotoxicity of O-Ethyl O-4-Nitrophenyl Phenylphosphonothioate: Subchronic (90 days) oral administration in hens. Toxicol. Appl. Pharmacol. 45: 685-700.

Abou-Donia, M.B., D.G. Graham, M.A. Ashry and P.R. Timmons (1980) Delayed Neurotoxicity of Leptophos and Related Compounds: Differential Effects of Subchronic Oral Administration of Pure, Technical Grade and Degredation Products on the Hen. Toxicol Appl. Pharmacol. 53: 150-163.

Abou-Donia, M.B. (1981) Organophosphorus Ester Induced Delayed Neurotoxicity. Ann. Rev. Pharmacol. Toxicol. 21: 511-548.

Abrahams, V.C., G.B. Koelle and P. Smart (1957) Histochemcial Demonstration of Cholinesterases in the Hypothalamus of the Dog. J. Physiol. (Lond.) 139: 137-144.

Ahmed, M.M. and P. Gless (1968) Ultrastructural Changes in Central Nerve Fiber of the Hen. Expl. Neurol. 22: 695-701.

Ahmed, M.M. (1973) The Ultrastructure of Tricresylphosphate Poisoning in Primates. 1. Studies on Axonal Alterations in the Spinal Cord. Arch. Histol. Jap. 35: 283-288.

Ahmed, M.M. and P. Gless (1977) Mitochondrial Degeneration after Organic Phospahte Poisoning in Prosimian Primates. Cell Tissue Res. 175: 459-465.

Ariens, A.T., E. Meeter, O.L. Wolthuis and R.M.J. Van Benthen (1969) Reversible Necrosis at the End-Plate Region in Striated Muscles of the Rat Poisoned with Cholinesterase Inhibitors. Experientia 25: 57-59.

Barnes, J.M. and F.A. Denz (1953) Experimental Demyelination with Organophosphorus Compounds. J. Pathol. Bacteriol. 65: 597-605.

Belcher, G., and R.W. Ryal (1977) Substance P and Renshaw Cells: A New Concept of Inhibitory Synaptic Interactions. J. Physiol. 272: 105-119.

Bischoff, A. (1970) The Ultrastructure of Tri-Ortho-Cresyl Phosphate Poisoning. II. Studies on Spinal Cord Alterations. Acta Neuropath. (Berl.) 15: 142-155.

Biscoe, T.J. and K. Krnjevic (1963) Chloralose and the Activity of Renshaw Cells. Exp Neurol. 8: 395-405.

Bouldin, T.W. and J.B. Cavanagh (1979) Organophosphorous Neuropathy. I. A teased-fiber study of the Spatio-Temporal Spread of Axonal Degeneration. Am. J. Pathol. 94: 241-252.

Bouldin, T.W. and J.B. Cavanagh (1979a) Organophosphorus Neuropathy. II. A Fine-Structural Study of the Early Stages of Axonal Degeneration. Am. J. Pathol. 94: 253-269.

Bradley, W.G. and M.H. Williams (1973) Axoplasmic Flow in Axonal Neuropathies. I. Axoplasmic Flow in Cats with Toxic Neuropathies. Brain 96: 235-246.

Brodeur, J. and K.P. Dubois (1964) Studies on the Mechanism of Acquired Tolerance by Rats to 0,0-Diethyl S-2-(Ethylthio)ethyl Phosphordithioate (Di-Syston). Arch. Int. Pharmacodyn. 149: 560-570.

Burt, A.M. (1971) The Histochemical Localization of Choline Acetyltransferase. Progr. Brain Res. 34: 327-335.

Burt, A.M. and A. Silver (1973) DFP as a Prerequisite in the Histochemical Demonstration of Choline Acetyltransferase. Brain Res. 57: 518-521.

Butcher, L.L. and L. Bilezikjian (1975) Acetylcholinesterase-Containing Neurones in the Neostriatum and Substantia Nigra Revealed after Punctate Intracerebral Injection of Di-isopropylflourophosphate. Eur. J. Pharmacol. 34: 115-125.

Butcher, L.L., K. Talbot and L. Bilezikjian (1975a) Acetylcholinesterase Neurones in Dopamine-Containing Regions of the Brain. J. Neural. Transm. 37: 127-153.

Butcher, L.L., R. Marchand, A. Parent, and L.J. Poirier (1977) Morphological Characteristics of Acetylcholinesterase-Containing Neurones in the CNS of DFP-Treated Monkeys. Part 3. Brain Stem and Spinal Cord. J. Neurol. Sci. 32: 169-185.

Cavanagh, J.B. (1954) The Toxic Effects of Tri-O-Cresyl Phosphate on the Nervous system. J. Neurol. Neurosurg. Psychiat. 17: 163-172.

Cavanagh, J.B. (1964) The Significance of the "Dying Back" Process in Experiemtnal and Human Neurological Disease. Int. Rev. Expl. Pathol. 3: 219-367.

Cavanagh, J.B. (1964a) Peripheral Nerve Changes in Ortho-Cresyl Phospate Poisoning in the Cat. J. Path. Bact. 87: 365-383.

Cavanagh, J.B. and G.N. Patangia (1965) Cnages in the central nervous system in the cat as the Result of Tri-O-Cresyl Phosphate Poisoning. Brain 88: 165-180.

Conradi, S. (1969) Ultrastructure and Distribution of Neuronal and Glial Elements on the Motoneurone surface in the Lumbosacral Spinal Cord of the Adult Cat. Acta Physiol. Scand. Suppl. 332: 5-48.

Conradi, S. (1976) Functional Anatomy of the Anterior Horn Motor Neurone. In: The Peripheral Nerve, edited by D.N. London. London: Chapman & Hall Ltd., 1976, pp. 279-329.

Couraud, J.Y. and L. DiGiamberardino (1980) Axonal Transport of the Molecular Forms of Acetylcholinesterase in Chick Sciatic Nerve. J. Neurochem. 35: 1053-1066.

Curtis, D.R., C.J.A. Game, D. Lodge, and R.M. McCulch (1976) A Pharmacological Study of Renshaw Cell Inhibition. J. Physiol. 258: 227-242.

Curtis, D.R., J.W. Phillis, and J.C. Watkins (1961) Cholinergic and Non-Cholinergic Transmission in the Mammalian Spinal Cord. J. Physiol. (lond.) 158: 296-323.

Davies, D.R. (1963) Neurotoxicity of Organophosphorus Compounds. IN: Handbuch der Experimentellen Pharmakologie; Eragaenzungswerk, Vol. XV; Cholinesterases and Anticolineserase Agents; Chapter 19, edited by G.B. Koelle. Berlin: Springer Verlag, 1963, pp. 860-882.

Davies, J., and A. Dray (1976) Effects of Enkephalin and Morphine on Renshaw Cells in Feline Spinal Cord. Nature 262: #5569, 603-604

Eccles, J.C., R.M. Eccles, and P.Fatt (1956) Pharmacological Investigations on a Central Synapse Operated by Acetylcholine. J. Physiol. (lond.) 131: 154-169.

Eccles, J.C., P. Fatt, and K. Koketsu (1954) Cholinergic and Inhibitory Synapses in a Pathway from Motor-Axon Collaterals to Motoneurones. J. Physiol. (Lond.) 126: 524-562.

Engel, A.G., E.H. Lambert, and T. Santa (1974) Study of Long-Term Anticholinesterase Therapy. Effects on Neuromuscular Transmission and on Motor End-Plate Fine Structure. Neurology 23: 1273-1281.

Fenichel, G.M., W.B. Kibler, W.H. Olson, and W.-D. Dettbarn (1972) Chronic Inhibition of Cholinesterase as a Cause of Myopathy. Neurology, 22: 1026-1033.

Fischer, G. (1970) Die Azetylcholinesterase an der Motorischen Endplatte des Rattenzwerchfells nach Intoxikation mit Paraoxon und Soman bei Applikatin von Oximen. Experientia 26: 402-403.

Frenton, J.C.B. (1955) The nature of the Paralysis in Chickens Following Organophosphorus Poisoning. J. Pathol. Bacteriol. 69: 181-189.

Glazer, E.J., T. Baker, and W.F. Riker, jr. (1978) The Neuropathology of DFP at Cat Soleus Neuromuscular Junction. J. Neurocytol. 7: 741-758.

Gless, P. and H. Janzik (1965) Chemically (TCP) Induced Fibre Degeneration in the Central Nervous System, with Reference to Clinical and Neuropharmacological Aspects. Progr. Brain Res. 14: 97-121.

Hasse, J. (1963) Die Transformation des Entladungsmusters der Renshaw-Zellen bei Tetanischer Antidromer Reizung. Pflugers Arch. Ges. Phsyiol. 276: 471-480.

Heuser, J.E. and T.S. Reese (1973) Evidence for Recycling of Synaptic Vesicle Membrane During Transmitter Release at the Frog Neuromuscular Junction. J. Cell Biol. 57: 315-344.

Hultborn, H., E. Jankowska, and S. Lindstrom (1971) Reccurrent INhibition of INerneurones Mono-Synaptically Activated from Group Ia Afferents. J. Physio. (lond.) 215: 613-636.

Illis, L., G.N. Patangia, and J.B. Cavanagh (1966) Boutons Terminaux and Tri-Ortho-Cresyl Phosphate Neurotoxicity. Expl. Neurol. 14: 160-174.

James, K.A.C. and L. Austin (1970) The Effect of DFP on Axonal Transport of Protein in Chicken Sciatic Nerve. Brain Res. 18: 192-194.

Johnson, M.K. (1975) The Delayed Neuropathy Caused by some Organophosphorus Esters: Mechanism and Challenge. CRC. Critical Rev. Toxicol. 3: 289 316.

Kaiya, H., G.W. Kreutzberg and M. Namba (1980) Ultrastructure of Acetylcholinesterase Synthesising Neurones in the Neostriatum. Brain Res. 187: 369-382.

Koelle, G.B. and A. Gilman (1949) Anticolinesterase Drugs. Pharmacol. Rev. 1: 166-216.

Koelle, G.B. (1954) The Histochemical localization of Cholinesterase in the Central Nervous System of the Rat. J. Comp. Neurol. 100: 211-235.

Krnjevic, K., and D. Lekic (1977) Post-Tetanic Potentiation and Depression of Renshaw Cell Discharges. Society of Neurosciences: 503. (Abstr.)

Laskowski, M.B., Olson, W.H. and W.-D. Dettbarn (1975) Ultrastructural Changes at the Motor End-Plate Produced by an Irreversible Cholinesterase Inhibitor. Expl. Neurol. 47: 291-306.

Laskowski, M.B., Olson, W.H. and W.-D. Dettbarn (1977) Initial Ultrastructural Abnormalities at the Motor End-Plate Produced by a Cholinesterase Inhibitor. Expl. Neurol. 57: 13-33.

Longo, V.G., W.G. Martin, and K.R. Unna (1960) A Pharmacological Study on the Renshaw Cell. J. Pharmacol Expl. Ther. 129: 61-68.

Lubinska, L. and S. Niemierko (1971) Velocity and Intensity of Bidirectional Migration of AChE in Transected Nerves. Brain Res. 27: 329-342.

McPhillips, J.J. (1969) Altered Sensitivity to Drugs Following Repeated Injections of a Cholinesterase Inhibitor to Rats. Toxicol. Appl. Pharmacol. 14: 67-73.

Parent, A., L.J. Poirier, R. Boucher and L.L. Butcher (1977) Morphological Characteristics of Acetylcholinesterase-Containing Neurones in the CNS of DFP-Treated Monkeys. Part 2. Diencephalic and Medial Telencephalic Structures. J. Neurol. Sci. 32: 9-28.

Pleasure, D.E., K.C. Mishler and W.K. Engel (1969) Axonal Transport of Proteins in Experimental Neuropathies. Science 166: 524-525.

Poirier, L.J., A. Parent, R. Marchand, and L.L. Butcher (1977) Morphological Characteristics of the Acetylcholinesterase-Containing Neurons in the CNS of DFP-Treated Monkeys. Part 1: Extrapyramidal and Related Structures. J. Neurol. Sci. 32: 181-198.

Preusser, H.-J. (1967) Die Ultrastruktur der Motorischen Endplatte im Zwerchfell der Ratte und Veraenderungen nach Inhibierung der Azetylcholinesterase. Z. Zellforsch. 80: 436-457.

Prineas, J. (1969) The Pathogenisis of Dying-Back Polyneuropathies. Part I: An Ultrastructural Study of Experimental Tri-Ortho-Cresyl Phospahte Intoxication in the Cat. J. Neurophathol. Expl. Neurol. 28: 571-597.

Reichert. B.L. and M.B. Abou-Donia (1980) Inhibition of Fast Axoplasmic Transport by Delayed Neurotoxic Organophosphorus Esters: A possible Mode of Action. Mol. Pharmacol. 17: 56-60.

Ross, H.-G., S. Cleveland, and J. Haase (1972) Quantitative Relation of Renshaw Cell Discharges to Monosynaptic Reflex Height. Pflugers Arch. 332: 73-79.

Russel, R.W., D.H. Overstreet, C.W. Cotman, V.G. Carson, L. Churchill, F.W. Dalglish and B.J. Vasques (1975) Experimental Tests of Hypotheses About Neurochemical Mechanisms Underlying Behavioral Tolerance to the Anticolinesterase, Diisopropylfluorophospate. J. Pharmacol. Expl. Ther. 192: 73-85.

Ryall, R.W. (1970) Renshaw Cell Mediated Inhibition of Renshaw Cells: Pattern of Excitation and Inhibition from Impulses in Motor Axon Collaterals. J. Neurophysiol. 33: 257-270.

Ryall, R.W., and M.F. Piercey (1971) Excitation and Inhibition of Renshaw Cells by Impulses in Peripheral Afferent Nerve Fibers. J. Neurophysiol. 34: 242-251.

Ryall, R.W., M.F. Piercey, and C. Polosa (1971) Intersegmental and Intrasegmental Distribution of mutual Inhibition of Renshaw Cells. J. Neurophysiol. 34: 700-707.

Shimizu, N. and S. Ishii (1966) Electron Microscopic Histochemistry of Acetylcholiesterase of Rat Brain by Karnovsky's Method. Histochemie 6: 24-33.

Somogyi, P. and I.W. Chubb (1976) The Recovery of Acetylcholinesterase Activity in the Superior Cervical Ganglion of the Rat Following its Inhibition by Diisopropylphosphorofluoridate: A Biochemical and Cytochemical Study. Neuroscience 1: 413-421.

Sterri, S.H., S. Lyngaas, and F. Fonnum (1980) Toxicity of Soman after Repetitive Injection of Sublethal Doses in Rat. Acta Pharmacol. et Toxicol. 46: 1-7.

Toth, L., S. Karcsu, G. Marosi, and Z. Simon (1980) Histochemische Analyse der Vielfaeltigen Wirkungen von Organophospaten (Wolfatox, DFP) bei Rattten. Acta Histocem. 66: 220-233.

VanMeter, W.G. (1978) Temporal analysis of post-tetanic depression of Antidromically Activated Renshaw Cells. Society for Neurosciences, Anaheim, Calif. (Abstr.)

VanMeter, W.G. and A.G. Karczmar (1978) CNS Effects of Anticholinesterases in the Presence of inhibited cholinesterases. Arch. int. Pharmacodyn. 231: 249-360

VanMeter, W.G., A.G. Karczmar, and R.R. Fiscus (1978) CNS effects of Anticholinesterases in the Presence of Inhibited Cholinesterases. Arch. int. Pharmacodyn. 231: 249-260.

DISTRIBUTION

4 Copies: Commander
US Army Medical Research and Development Command
ATTN: SGRD-RMS
Fort Detrick
Frederick, MD 21701

5 Copies: Commander
US Army Medical Research and Development Command
ATTN: SGRD-PLE
Fort Detrick
Frederick, MD 21701

12 Copies: Administrator
Defense Technical Information Center
ATTN: DTIC-DDA
Cameron Station
Alexandria, VA 22314

1 Copy: Commandant
Academy of Health Sciences, US Army
ATTN: AHS-CDM
Fort Sam Houston, TX 78234

1 Copy: Dean, School of Medicine
Uniformed Services University
of the health Sciences
4301 Jones Bridge Road
Bethesda, MD 20014